Sudden Arrhythmic Death Syndrome in the UK
The experience of a tertiary cardiogenetics centre in South London

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ABSTRACT

Background

In a proportion of sudden cardiac deaths (SCD), no structural pathology can be identified and a diagnosis of sudden arrhythmic death syndrome (SADS) is advocated. Recognition of SADS is important since a significant proportion is attributed to inherited cardiac conditions, and evaluation of relatives may identify individuals at risk.

Aims

The aims of the thesis are: To investigate the magnitude of SADS in young (≤35 years) and athletic individuals; To investigate the impact of expert cardiac pathology; To evaluate a large cohort of SADS families to ascertain: 1.The diagnostic yield of familial evaluation, 2.Short-term outcomes, 3.The implications of autopsy findings of uncertain significance, 4.The impact of higher intercostal leads, 5.The limitations of current risk stratification protocols in Brugada syndrome (BrS).

Methods and Results

Appraisal of the Office of National Statistics data revealed an incidence of young SADS of 0.24/100,000/year. Histopathological evaluation of hearts of athletic individuals who experienced SCD (n=118), identified a morphologically normal heart in 23% of cases. A study of 158 SCD cases where a pathological evaluation was performed by both the referring and an expert cardiac pathologist identified a disparity in the diagnosis in 41% of cases.

Evaluation of 83 families of victims of SADS demonstrated a diagnostic yield of 48%, with BrS being the predominant familial diagnosis. A quarter of the relatives (65 of 271

evaluated) were diagnosed with an inherited condition. During 25.5±16.8 months of follow-up, 1 patient died, despite being cleared after comprehensive clinical evaluation. A review of 50 SADS victims with a familial diagnosis of BrS identified high-risk features in only 20%, highlighting the limitations of current risk stratification protocols. Utilising higher intercostal V1 and V2 leads during Ajmaline testing doubled the diagnostic yield of BrS. Finally, familial evaluation following SCD with autopsy findings of uncertain significance identified a similar proportion of primary arrhythmogenic syndromes to “true” SADS.

Conclusions

After a suspected SADS death expert cardiac pathology evaluation is necessary to ensure accurate diagnosis. First-degree relatives should be referred for comprehensive cardiac screening in an expert setting. Higher intercostal leads should be used when BrS is suspected and there is a need for improved risk stratification protocols in BrS.

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DEDICATION

To my parents, without their continuous and unconditional support I would have never materialized my dreams.

To my wife, who supported me throughout my research and is my incessant source of inspiration.

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INTRODUCTION TO THE THESIS

Sudden cardiac death (SCD) accounts for 50% of cardiovascular mortality with an estimated annual toll between 100,000 and 120,000 deaths in the United Kingdom (UK).[1,2] The majority of SCDs are secondary to atherosclerotic coronary artery disease and affect the older segment (>35 years) of the population.[3] Occasionally, a young (≤35 years), apparently healthy person falls victim. The majority of SCDs in the young are secondary to previously quiescent, inherited cardiac diseases, galvanizing discussions relating to primary and secondary prevention strategies to avert such catastrophes. In a significant proportion of sudden deaths no obvious cause is identified and the death is attributed to "natural causes".[4,5] Failure to identify a cause of death may be attributed to inherent limitations of the autopsies, as autopsies performed at the request of the coroner, are predominantly geared to rule out foul play. It is well established however, that in a proportion of cases no cause is identified despite detailed histopathological examination and toxicology screen by a cardiac pathologist.[6] In such cases the death is presumed to be secondary to primary arrhythmia and is classified as sudden arrhythmic death syndrome (SADS).[7]

A number of primary prevention strategies have been proposed to avert SCD in the young ranging from targeted evaluation of high-risk individuals, such as people with symptoms suggestive of cardiac disease or family history of inherited cardiac conditions or SCD, to widespread screening of all young individuals and in particular young athletes, irrespective of background risk. The use of automated external defibrillators (AED) in public venues has been recommended for secondary prevention. As part of the National service framework for coronary heart disease, the department of health published in 2005 the chapter 8 for arrhythmias and SCD, endorsing targeted screening. The authors recognised

that when sudden unexplained cardiac death occurs, close relatives are at potential risk of having a fatal cardiac condition and that a coroner's post-mortem is vital to determine the cause of death and provide the opportunity to assess the potential risk to the family. They advocated, and proposed as markers of good practice, that an expert post mortem is carried out and evaluation of relatives takes place in a dedicated clinic, with staff who are trained in the diagnosis, management and support for these families.[8]

The implementation of preventative strategies is hindered by the considerable lack of evidence in the field of SCD, particularly in young individuals. The epidemiology of SCD, and SADS, is not well established in the young due to the absence of systematic registries. Moreover, there is no evidence to support the added value of cardiac pathology for the post-mortem evaluation of victims of SCD compared to the routine coroner's autopsies. Finally, there are limited studies assessing the impact of targeted familial evaluation in an expert setting, and questions relating to the best investigative protocol remain unanswered.

The aim of the current thesis was to offer a comprehensive review of SADS, incorporating epidemiological data, with the group's experience from a specialist cardiac pathology unit and a specialist inherited cardiac diseases clinic. Given the complexity of the project, the large volume of data required, and the relatively low frequency of conditions predisposing to SADS, collaboration with leading institutions and individuals with established expertise in relevant areas was essential. To ensure the success of the project the author reinforced collaborative links between Prof Sharma's inherited cardiac diseases clinic in Lewisham and Dr E.R Behr at St George's hospital, Prof M.N Sheppard at Royal Brompton hospital, the clinical genetics department at St George's hospital and the cardiac MRI unit at Royal Brompton hospital.

The primary aim was to assess the diagnostic yield of comprehensive clinical evaluation in an expert setting of a large cohort of SADS families. Additional aims were to ascertain the effect of familial evaluation to short-term outcomes and investigate the impact of utilizing higher intercostal leads in the diagnosis of Brugada syndrome (BrS), a fairly novel primary arrhythmogenic syndrome implicated in SADS. Adopting a holistic approach, the author also aimed to investigate the magnitude of SADS in young and athletic individuals and explore the impact of cardiac pathology. Based on the author's experience during the project, two further, novel research questions were developed; 1. What is the significance and implications of autopsy findings that suggest cardiac structural abnormalities but are not diagnostic of conditions implicated in SCD (uncertain significance)? and 2. How effective would current risk stratification protocols in Brugada syndrome (BrS) have been in identifying the SADS probands that initiated familial evaluation in our clinic?

To investigate the magnitude of SADS in the young, in chapter 1 the authors critically appraised data from the ONS for causes of death in the 1-34 years age group in England and Wales. To investigate the impact of SADS in athletic individuals in chapter 2 the authors retrospectively analysed 118 hearts of people who participated in regular sport activities, referred to the Centre for Cardiac Pathology in the National Lung and Heart Institute. In chapter 3, we evaluated a sample of 158 consecutive cases of SCD, where both the referring pathologist and the cardiac pathologist examined the heart and provided a cause of death, in order to assess diagnostic discrepancies between general and specialist pathologist and potential implications. In chapter 4, the authors evaluated a large cohort of SADS families to ascertain the diagnostic yield of familial evaluation and short-term outcomes. In chapter 5 we were able to ascertain the implications of autopsy findings of uncertain significance by correlating post-mortem with clinical data. Finally, by utilizing

data from the cohorts in chapters 4 and 5, the author investigated the impact of higher intercostal leads (chapter 6) and the limitations of current risk stratification protocols in Brugada syndrome (BrS) (chapter 7).

Chapter 1: The epidemiology of sudden cardiac death and sudden arrhythmic death syndrome in the young

(Publication attached in appendix 3)

1.1 Introduction

The epidemiology of SCD in the young (≤35 years) is not well established. Studies in young athletic and non-athletic individuals have reported a wide range of incidence of SCD ranging from 0.5 per 100,000 per year to 11 per 100,000 per year (Table 1).[9,10] Most studies report a male predominance with a male to female ratio of 3:1 in the general population and 9:1 in young athletes.[11] Inherited cardiomyopathies are the commonest cause of SCD in athletes, whereas coronary artery pathology including coronary artery anomalies and atherosclerotic disease predominate in the non-athletic population.[10,12-14]

1.1.1 The Olmsted County study

One of the most robust studies investigating sudden death in young individuals is the Olmsted County study.[12] The investigators collected prospective data on all sudden, unexpected, non-traumatic deaths of residents aged 20–40 years over a 30-year period. Data collection included death certificates, complete community outpatient and inpatient medical records, coroner's reports, and autopsy reports. Most importantly, all individuals included in the study had undergone post-mortem evaluation, including histological

evaluation and toxicology screen, at a single centre and in particular in the Pathology Department of the Mayo Clinic. The incidence of sudden death was estimated based on population census data.

Table 1: Summary of studies reporting the incidence of SCD and SADS in different populations in the order discussed in the thesis.

Country [Ref] (Region)	Studied population	Nature of study	Time period	Age in years	Incidence of SCD (SADS) per 100,000
USA[12] (Olmsted county)	General	Prospective	1960-1989	20-40	3.6 (0.6)
USA[10]	Military recruits	Retrospective	1977-2001	18-35	11.2 (4.6)
USA[15]	Military personnel	Retrospective	1998-2008	18-35	3.5 (1.2)
Sweden[16]	General	Retrospective	1992-1999	15-35	0.93 (0.20)
Denmark[17]	General	Retrospective	2000-2006	1-35	2.8 (0.81)
Ireland[18]	General	Retrospective	2005-2007	14-35	2.9 (0.76)
UK[4] (Wandsworth)	General (Caucasian)	Prospective	3 years published in 1988	18-69	37.3 (1.83)
UK[3]	General (Caucasian)	Prospective	1993-1995	16-64	10.5 (0.50)
England[7]	General (Caucasian)	Prospective	1997-1999	4-64	---- (0.16)
USA[9] (Minnesota)	High school athletes	Retrospective	1985-1997	15-17	0.46 (0)
USA[19]	High school & college athletes	Prospective	1983-1993	13-24	0.75 (0.04)
USA[20]	High school athletes	Retrospective	2006-2007	14-17	4.4 (----)
USA[21]	College athletes	Retrospective	2004-2008	17-23	2.3 (----)
USA[14]	Athletes	Prospective	1980-2006	8-39	0.61 (----)
Italy[22] (Veneto)	Athletes	Prospective	1979-2004	12-35	1.9 (----)

Based on the identification of 54 deaths between 1960 and 1989 the incidence of sudden, unexpected, non-traumatic death was estimated to be 6.2 per 100,000 per year. The majority of deaths (57%) were attributed to cardiac causes, with an estimated incidence of SCD of 3.6 per 100,000 per year. The predominant cause of SCD (18 of 31 cases; 58%) was coronary artery disease defined as post-mortem evidence of severe chronic obstructive atherosclerosis, acute plaque rupture, acute thrombotic occlusion and acute myocardial ischaemia. Other cardiac pathology included presumed primary arrhythmia (n=6) and myocarditis (n=4). On initial inspection the study appears to suggest that cardiomyopathy and in particular hypertrophic cardiomyopathy (HCM) accounted for only 2 deaths. Detailed review of the deceaseds' characteristics, however, reveals that in 9 cases the histological findings were consistent with a diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC), defined by the authors as interstitial fatty infiltration extending through 75% of the RV myocardial thickness. Of the 9 deaths, 3 were attributed to seizures, 2 to coronary artery disease, 2 to unknown cause, 1 to arrhythmia and in 1 case there was evidence of associated aortic dissection. Of importance, 7 deaths were ascribed an unknown cause of death and in 5 of these cases the post-mortem was reported as normal. Adding individuals with possible ARVC, in the absence of histological evidence of other cardiac pathology (n=6) and those with a normal post-mortem, which could represent SADS deaths (n=5), would bring the incidence of SCD to 4.8 per 100,000 per year and SADS to 0.6 per 100,000 per year.

1.1.2 Studies in military personnel

The military personnel provide a unique opportunity to examine the incidence and causes of SCD. Unlike studies in the general population, the exact number of persons under examination is usually clearly identifiable, health care provision is captured and

documented and there is centralization of autopsy reporting. Active surveillance in health and in death, with routine performance of an autopsy, allows for reduction of case referral bias, as might be seen in high-profile cases with disproportionate media attention.[10,15,23,24] On the other hand however, by virtue of a military population, there is risk of ascertainment bias. Moreover, results from the military population do not necessarily extrapolate to the general population, given that they represent a fitter, almost athletic population as a result of mandated weight control and physical fitness standards.

A 25-year review of autopsies in United States (US) military recruits aged 18–35 revealed a high rate of non-traumatic sudden deaths of 13 per 100,000 recruit-years.[10] The authors utilized census data relating to recruit numbers and data held from the Department of Defense Recruit Mortality Registry. They retrospectively analysed all recruit deaths between 1977 and 2001. The authors identified 277 deaths among 6.3 million recruits. Of those, 152 were non-traumatic and in the great majority (n=148; 97%) an autopsy report was available. One hundred and twenty-six deaths were judged to represent a sudden death defined as an event resulting in death or terminal life support within 1 hour of the inciting event and were included in the study. Half of the deaths (n=64; 51%) were defined as cardiac in origin based on the pathological findings and the clinical circumstances. As in the Olmsted County study, the most prevalent pathology was related to coronary artery disease (n=39; 61% of cardiac deaths) but on this occasion coronary artery anomalies rather than coronary artery atherosclerosis was the predominant finding.[24] Myocarditis (n=13) and cardiomyopathies (HCM: n=8; ARVC: n=1) accounted for most of the remaining cardiac deaths. Most importantly, however, 44 of the 126 sudden deaths (35%) were labeled as idiopathic based on the absence of pre-existing disease and a normal post-mortem examination. Such deaths are likely to represent deaths secondary to an arrhythmic event and would be classified as SADS deaths by contemporary criteria,

representing an incidence of 4.6 per 100,000 recruit-years. Should we add the potential SADS deaths to the cardiac deaths reported by the authors, the estimated rate of SCD would increase to 11.2 per 100,000 recruit-years, which is the highest rate reported in the literature.

In the limitations of the study the authors highlight that the reported incidence may represent an underestimate since all recruits undergo pre-enlistment screening with history and cardiovascular examination, which may have identified individuals with cardiac disease and prevented a number of sudden deaths. Other limitations of the study relate to the fact that post-mortem evaluations were performed by a number of pathologists in military and general hospitals with no consistent diagnostic criteria and, therefore, the accuracy of the autopsy conclusions may have been compromised. Finally, almost one-third (27%) of victims classified as idiopathic sudden death were associated with sickle-cell trait, which according to recent publications may in its own right predispose to sudden death.[25,26]

In a study published in 2011 by the same group, the authors reviewed all deaths that occurred in uniformed personnel, including recruits as in the previous study, while on active duty. All death records were reviewed by at least 3 authors, on this occasion, and a final determination was made as to clinical cause of death. Of 14,771 deaths between 1998 and 2008, 902 deaths were thought to represent non-traumatic, sudden deaths of potentially cardiac aetiology for which complete data, including an autopsy report were available. Of the 902 deaths, 298 deaths affected young individuals defined as <35 years, accounting for an incidence of SCD in the young close to 3.5 per 100,000 person-years, which is considerably lower than the reported incidence in military recruits. Unfortunately, the authors did not provide an explanation for this discrepancy. Although the consistency

of the population may have accounted to some extent for the difference, it is unlikely that it justified such a disparity. Military personnel who are under regular medical surveillance and are subjected to regular fitness tests over a number of years may be less likely to exhibit significant cardiovascular disorders, compared to newly recruited individuals who are more representative of the general population. Coronary artery pathology was the predominant cause of structural heart disease (n=88; 30%), followed by cardiomyopathy (n=67; 22%) and myocarditis (n=17; 6%). In this cohort however, the most common cause of potential SCD in the young was SADS, with 123 (41%) of victims classified as sudden unexplained death based on the absence of any significant ante-mortem history and a normal post-mortem examination. Based on the census data the estimated incidence of SADS was 1.2 per 100,000 per year in individuals less than 35 years old and 2.0 per 100,000 per year in older recruits.

1.1.3 Retrospective general population reviews

Winsten et al. investigated the incidence of SCD in young individuals between the ages of 15 and 35 years in Sweden.[16] The authors reviewed a National forensic medicine database for deaths between January 1992 and December 1999. Most unexpected, sudden deaths in Sweden undergo a post-mortem, which according to the authors are conducted by a small number of pathologists, adhering to established protocols. One hundred and eighty-one deaths were identified, corresponding to an overall incidence of SCD of 0.93 per 100,000 per year. Cardiomyopathies were the most common reported pathology, accounting for 29% of all cases (DCM 12%, HCM 10%, ARVC 7%). A structurally normal heart, corresponding to SADS deaths, was present in 21% of cases. Coronary atherosclerosis was considered the principal cause of death in 18% of the cases but the authors failed to provide details relating to the severity of the coronary artery

lesions and as to whether evidence of acute or previous myocardial infarct were present. The authors identified a gender predilection with a male to female ratio of almost 3:1. The reported incidence of SCD is likely to represent an underestimate given that comparison with the Swedish Death Registry, a central database where all deaths are recorded irrespective of forensic post-mortem examination, revealed that 20% of potential SCDs had not been included in the forensic database.

More recently, nationwide death certificate and autopsy reports based reviews performed in Ireland and Denmark reported an incidence of SCD of 2.9 per 100,000 person-years and 2.8 per 100,000 person-years, respectively.[17,18] Both studies were published after the commencement of the current thesis and after the publication of our Office of National Statistics data and utilized similar methodology. In the Irish study the investigators reviewed all deaths in individuals aged between 15 and 35 years. Utilizing death certificates, the authors identified 292 cases that could possibly represent SCD. Of those 116 cases (40%) were included in the study analysis based on the presence of an adequate post-mortem, which in the authors' opinion confirmed cardiac pathology. The predominant cause of death was SADS (27%), based on a normal post-mortem. This represented an incidence of SADS of 0.76 per 100,000 per year, although the exclusion of the paediatric population may have resulted in an underestimate. Other prevalent causes included coronary artery disease with evidence of acute or prior myocardial infarction (21%) and cardiomyopathies (19%). Of importance, this study differentiates between HCM and left ventricular hypertrophy (LVH) based on the presence of myocardial disarray, with LVH accounting for 10% of SCDs.

In the Danish study the authors reviewed death certificates of individuals aged 1-35 years. Of the 470 classified as SCDs, 314 (67%) had undergone a post-mortem. In the autopsied

cases, the most prevalent finding was that of a normal heart (43%), implying a SADS death. Based on census data the incidence of SADS was estimated at 0.81 per 100,000 per year. Ischaemic heart disease accounted for 13% of deaths. A definitive diagnosis of cardiomyopathy was present in 22 (7%) of autopsied cases with ARVC being the most common one. In a further 34 cases, the post-mortem reported a hypertrophied heart or myocardial fibrosis. Sudden cardiac death exhibited a gender predilection with a male to female ratio of 2:1. There was also an age predilection; for every decade, the risk of SCD roughly doubled in relation to the previous decade and the risk of dying of SCD was >10 times higher for persons aged 30–35 years than for persons aged 1–10 years.

1.1.4 The epidemiology of sudden arrhythmic death syndrome in the United Kingdom

In the UK the magnitude of SADS in the general population has been studied specifically or as part of the wider spectrum of sudden death in three prospective coroners' studies. The Wandsworth study, published back in 1988, reported on post-mortem findings of Caucasian individuals aged between 18 and 69 years who experienced a sudden death of "natural" causes.[4] The authors prospectively monitored all non-traumatic deaths over a 3-year period. All individuals underwent extensive post-mortem examination and toxicology screen, according to well-defined protocols. The authors outlined clear diagnostic criteria for cardiac pathology, including strict criteria for the diagnosis of ischaemic heart disease. Of the 322 deaths included in the study, 224 (70%) deaths were thought to be secondary to SCD. Ischaemic heart disease accounted for the majority of sudden cardiac deaths (84%) while in a small but significant proportion of potential sudden cardiac deaths (4.9%) no cause was identified despite detailed histopathological examination. Based on census data, these figures equated to a SADS incidence of 1.83 per 100,000 per year.[4]

The first national survey of SCD and SADS in the UK was published in 2003.[3] The authors performed a prospective survey using a stratified random sample of 83 coroners of the 132 coroner's jurisdictions in England. The total period of surveillance was about 2 years. Subjects included in the study were apparently healthy, Caucasian individuals, aged 16-64 years, who died suddenly. Information collected by the investigators included pre-morbid history, circumstances of death and the results of the local post-mortem examination. A transverse myocardial slice (1 cm thickness) through both ventricles at mid-septal level and blood sample was sent to the investigators. In those cases in whom macroscopic inspection of the transverse myocardial slice revealed no evidence of infarction or ischaemia and in whom neither significant coronary disease nor any other identifiable cause of death was found at post mortem, the whole heart was sent to the investigators for examination. Of the 692 cases of SCD identified, in 528 cases the authors had the opportunity to evaluate a complete set of data. The study's expert cardiac pathologist examined 517 myocardial slices and 47 whole heart specimens. Out of the 564 cases where tissue samples were reviewed by the expert cardiac pathologist, 4.1% had a morphologically normal heart, with negative toxicology. Based on the estimated age-specific population census data the incidence of SADS was estimated at 0.5 per 100,000 per year.[3]

The same group performed a further national prospective study surveying 117 coroner's jurisdictions in England over a 20-month period. They attempted to identify all SADS deaths in Caucasian individuals aged 4-64 years. During the same period the authors reviewed the Office of National Statistics (ONS) mortality figures for certified causes of death in 4-64-year-olds in England. The investigators identified deaths ascribed to the International Classification of Diseases-ninth edition (ICD-9) code 798.1 (sudden death cause unknown-instantaneous death) which is considered to most accurately represent a

SADS death, as well as other ICD-9 codes that were likely to represent SADS deaths. The study reported an estimated SADS mortality of 0.16 per 100,000 per year based on the coroner survey compared to 0.10 per 100,000 per year estimated by the 798.1 ICD-9 code of the ONS data. This considerable underestimate was a result of misclassification of SADS deaths identified by the coroner's survey under other ICD-9 codes including myocardial infarction, cardiomyopathy, epilepsy, drowning and other ill-defined causes.[7] Inclusion of other ICD-9 codes for ill-defined and unknown causes of mortality to which SADS cases could be inaccurately attributed, increased the potential annual death rate for SADS as high as 1.38 per 100,000 per annum.

Based on the estimated incidence of potential SADS from the ONS data, the authors noted an age predilection. The incidence of SADS was lower in the very young (0.14 per 100,000 per year for individuals aged 4-15 years) and gradually increased and peaked at 2.36 per 100,000 per year in individuals aged 45-54 yeas. Additionally, although there was no significant gender predilection in the very young (4-15 years), the incidence of SADS gradually increased in males with advancing age, with a peak male to female ratio of 3.2 to 1 in the fourth decade.

1.2 Aim

The incidence of SADS in young individuals has not been studied and in the absence of a systematic national registry documenting sudden cardiac deaths in the young, the true impact of such fatalities in the UK is speculative. Unsurprisingly, the sudden death of a young individual commonly galvanises emotionally charged debates between the lay and medical communities relating to the demand for preventative measures, underscoring the need to establish the magnitude and pathogenesis of sudden cardiac deaths in the

young.[11,27] Such information is fundamental to facilitate any debate about local provisions for a potential cardiovascular screening programmes and subsequent exercise recommendations.[28-30] To address the scientific gap relating to the magnitude of SADS in young individuals in the UK the authors examined and critically appraised the Office of National Statistics data for causes of death in the 1-34 years age group in England and Wales for four consecutive years, (2002-2005). We estimated the incidence of SCD and SADS, examined gender and age distribution and attempted to assess the impact of potential misclassifications of causes of death.[31]

1.3 Personal contribution

The author collected, reviewed and analysed all ONS data relevant to the project and drafted the published manuscript.

1.4 Methods

The ONS is the government agency responsible for compiling, analysing, and disseminating many of the National statistics including periodic census of the population and health statistics. The data used in the mortality statistics are derived from information obtained by the doctor certifying the death, the coroner, and details supplied by the informant to the Registrar. The deaths in males and females are reported by the ONS in 5-yearly age groups. The causes of death are registered according to the International Classification of Diseases-10 (ICD-10).[32]

This study was based purely on available data from the ONS and the author did not directly review death certificates or post-mortem reports. The investigators analysed the

ONS mortality data stating the cause of death for England and Wales for four consecutive years 2002–2005, inclusive. Five of the ICD-10 chapters were included in the analysis (Table 2). Within these chapters, two of the senior investigators (Dr Elijah R. Behr. and Prof M.N. Sheppard) scrutinized the ICD-10 classification codes to identify codes that may represent cardiac deaths. Data were then summed from the existing age subgroups to include deaths of individuals from the age of 1 year to the age of 34 years, inclusive.

Table 2: List of the 5 chapters of the World Health Organisation International Classification of Diseases – 10 included in the analysis	
Chapter VI	Diseases of the nervous system
Chapter IX	Diseases of the circulatory system
Chapter X	Diseases of the respiratory system
Chapter XVIII	Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified
Chapter XX	External causes of morbidity and mortality

The selected ICD-10 codes were subsequently divided into four classes as deemed relevant by the investigators (Table 3): Class A1: definite cardiac deaths with no structural heart disease identified at post-mortem representing SADS; Class A2: definite cardiac deaths with structural heart disease identified at post-mortem comprising sudden and non-sudden deaths with likely causation by structural heart disease; Class A3: definite cardiac deaths with indeterminate cause comprising sudden and non-sudden deaths where the presence or absence of underlying heart disease was either not recorded or ill defined; and Class B: possible cardiac deaths since a proportion of these deaths may represent misclassifications of cardiac deaths and in particular SADS as epilepsy or drowning. Where there was disagreement relating to the class of an ICD-10 code, a third senior author (S.S.) was consulted. Although the great majority of deaths referred to “natural causes” (non-accidental, non-malicious), a small proportion of the total cohort is likely to represent accidental deaths since ICD-10 codes W65–W74 from Chapter XX representing accidental drowning and submersion were included in Class B.

Incidence rates were calculated based on the ONS census data of the resident population for individuals aged 1–35 years in England and Wales. Data were further analysed according to age subgroup and gender in order to identify potential trends or gender differences.

Table 3: Examples of the most frequent ICD-10 codes included in each Class (presented in order of frequency)

Class	ICD-10 code
Class A1 (definite cardiac deaths with NO structural disease)	R96: Other sudden death, cause unknown
	I49.9: Cardiac arrhythmia, unspecified
	I46.1: Sudden cardiac death, so described
	I45.6: Pre-excitation syndrome (WPW)
Class A2 (definite cardiac deaths with structural disease)	I21.9: Acute myocardial infarction, unspecified
	I25.1: Atherosclerotic heart disease
	I42.0: Dilated cardiomyopathy
	I42.9: Cardiomyopathy, unspecified
Class A3 (definite cardiac deaths with indeterminate cause)	I50.9: Heart failure, unspecified
	I51.9: Heart disease, unspecified
	I50.1: Left ventricular failure
	I50.0: Congestive heart failure
Class B (possible cardiac deaths)	G40.9: Epilepsy, unspecified
	G41.9 Status epilepticus, unspecified
	W69 Drowning and submersion while in natural water
	J46 Status asthmaticus

Ethical approval

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Statistical analysis

Data interpretation and analyses were performed using SPSS software, version 14 (SPSS

Inc., Chicago, IL, USA). Data are expressed in means and standard deviations. Annual mortality incidence per 100,000 was calculated as the mean of the 4 years using the following type: (100 000 x number of deaths)/population size. Chi-square or Fisher's exact test was used to test group differences of proportions.

1.5 Results

The number of deaths in the 1–34 years age group is reported in table 4 according to class (A1, A2, A3, B) and year of death. Analysis of the ONS data revealed an average of 419±16.5 definite cardiac deaths per annum (Class A1 + A2 + A3) equating to 8 young cardiac deaths per week in England and Wales. On the basis of the average estimated size of the resident population of this age of 23,564,050, these data indicate an incidence of young cardiac death of 1.8±0.1 per 100 000 per year. There were also an average of 433±6.2 deaths per year, also corresponding to about 8 deaths per week, in Class B which comprised primarily deaths from drowning, epileptic seizures, and other ill-defined causes of mortality (Figure 1). There was no significant variation in the number of the resident population or the number of deaths per year.

Table 4: Number of deaths according to class per year

Class	No. of deaths per year				Total No of deaths	Mean deaths per annum (SD)	Mean mortality rate/100,000/ annum (SD)
	2002	2003	2004	2005			
A1	60	50	57	61	228	57.0 (5.0)	0.2 (0.0)
A2	363	358	324	324	1369	342.3 (21.2)	1.5 (0.1)
A3	19	20	21	20	80	20.0 (0.8)	0.2 (0.0)
A1+A2+A3	442	428	402	405	1677	419.3 (19.1)	1.8 (0.1)
B	438	424	434	436	1732	433.0 (6.2)	1.8 (0.0)
Total (A1+A2+A3 +B)	880	852	836	841	3409	852.3 (19.7)	3.6 (0.1)

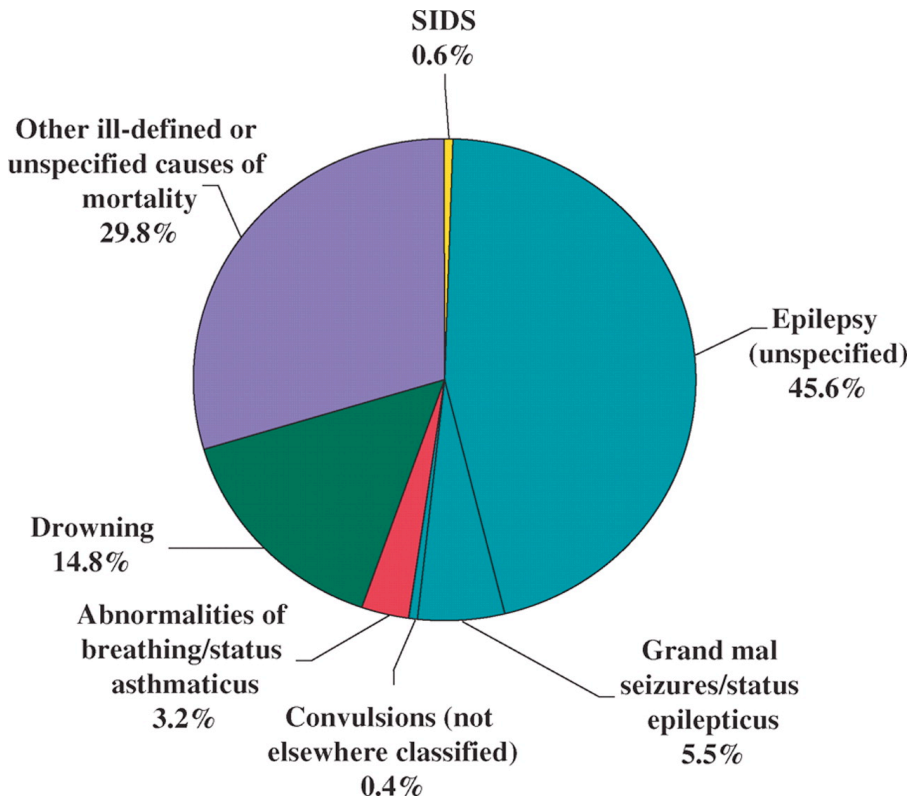


Figure 1: Causes of death in the young expressed as percentage of the total number of deaths in Class B (possible cardiac deaths).

SIDS: sudden infant death syndrome.

The most prevalent cardiovascular pathology identified in ONS was ischaemic heart disease comprising one-third (33.5%) of the definite cardiac deaths (A1 + A2 + A3). Although the majority of ischaemic deaths (56%) were attributed to acute myocardial infarction (ICD-10 code: I21.9), in a significant proportion (32%), the presence of atherosclerotic heart disease alone (ICD-10 code: I25.1) was documented as the principal cause of death, comprising 19% and 11% of the definite cardiac deaths, respectively (Figure 2). Cardiomyopathies were the second commonest cause of cardiac death corresponding to 27% of definite cardiac deaths, with dilated and hypertrophic cardiomyopathies accounting for 12% and 5% of the deaths, respectively. Sudden arrhythmic death syndrome accounted for 14% of definite cardiac deaths followed by

myocarditis (11%), valvular heart disease (5%), and hypertensive heart disease (2%) (Figure 2).

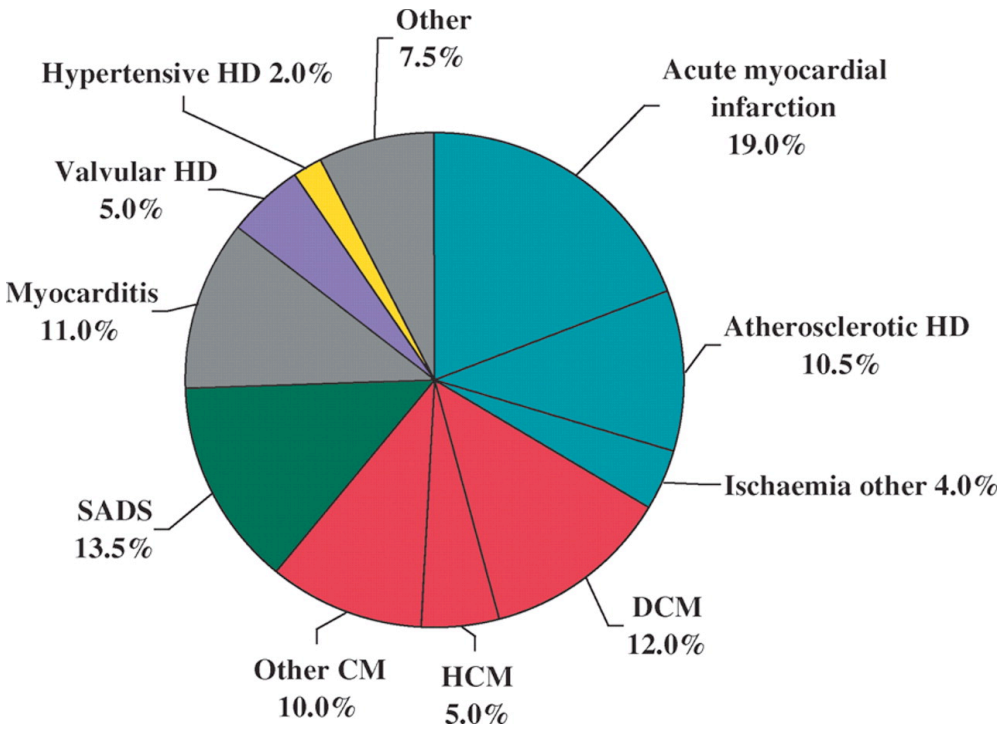


Figure 2: Causes of cardiac death in the young expressed as percentage of the total number of definite cardiac deaths (A1 + A2 + A3).

DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy; HD: Heart disease; CM: Cardiomyopathy; SADS: Sudden arrhythmic death syndrome.

1.5.1 Causes of death by gender and age

Definite cardiac deaths (Class A1 + A2 + A3) were more prevalent among males with a male to female ratio of 2.4 : 1. The only pathology associated with a statistically significant difference between the male and female gender was ischaemic or potentially ischaemic causes which accounted for 22% of all deaths in males but only 13% in females ($p<0.001$). The same gender difference was also observed for possible cardiac deaths (Class B) with a male to female ratio of 2.0 : 1. Deaths attributed to epilepsy accounted for 22% of all

deaths in males but 31% in females (p<0.001), whereas drowning-related deaths were more prevalent among males (9% vs. 4%, p<0.001) (Figure 3).

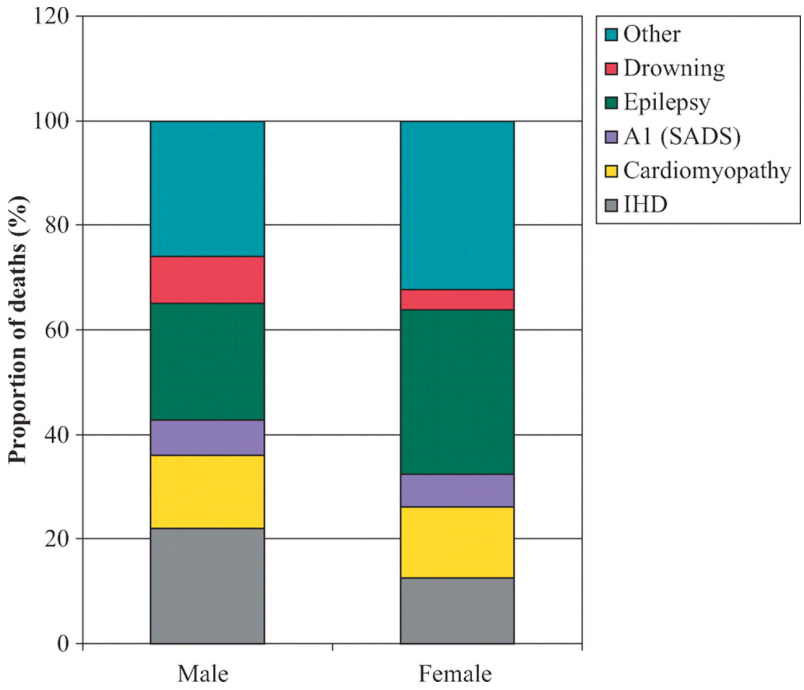


Figure 3: Proportional (%) distribution of underlying cause of death by gender.

IHD: Ischaemic heart disease; SADS: Sudden arrhythmic death syndrome.

There was a rising incidence of definite cardiac deaths with advancing age, with individuals ≥30 years old having a 10-fold risk compared with children aged <10 years. The only underlying causes of definite cardiac death exhibiting a significant age trend were ischaemia and cardiomyopathies. Ischaemic deaths exhibited an increasing trend with advancing age, accounting for almost one-third (31%) of all deaths in the 30–34 years age group but only 1% in children aged <10 years (p<0.001). In contrast, cardiomyopathy-related deaths peaked during adolescence, accounting for a greater proportion of deaths at the 10–19 years age group (18%) and accounting for only 11% of deaths in the 30–34 years age group (p=0.001) (Figure 4). Deaths secondary to SADS did not exhibit any significant gender or age predilection.

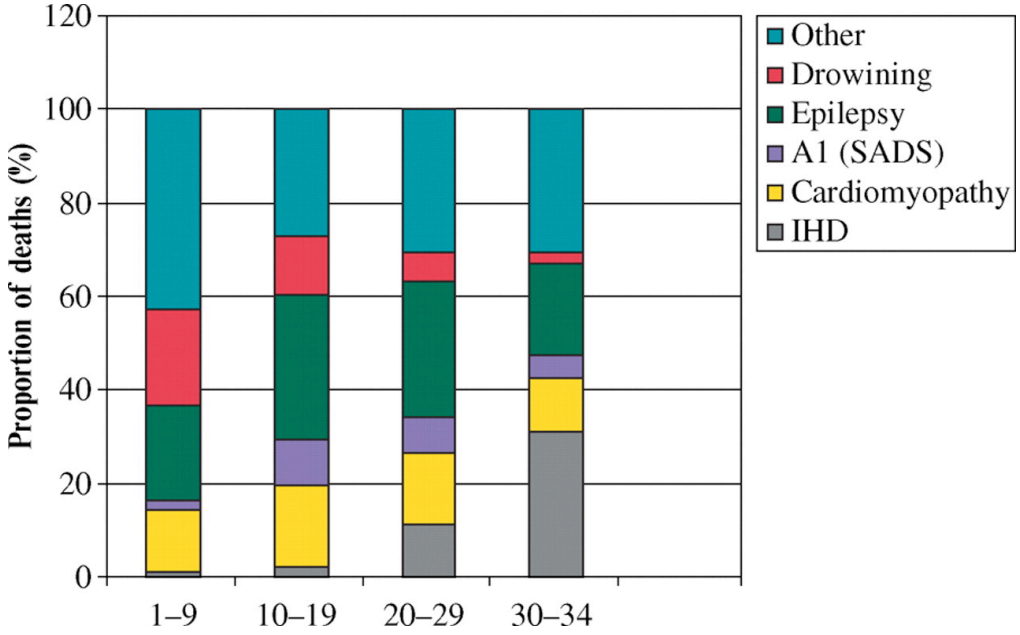


Figure 4: Proportional (%) distribution of underlying cause of death by age.

IHD: Ischaemic heart disease; SADS: Sudden arrhythmic death syndrome

A similar trend to definite cardiac deaths (A1-3) was observed with possible cardiac deaths in Class B, with individuals 30–34 years old having a five-fold risk compared with children aged <10 years old. Underlying causes exhibiting a statistically significant age trend included deaths attributed to epilepsy and drowning. Epilepsy-related deaths peaked in the second and third decades of life, accounting for almost one-third of deaths in the 10–19 and 20–29 years age groups ($p<0.001$). Drowning exhibited a reverse trend with age, accounting for 21% of deaths in children aged <10 years but only 3% in individuals ≥30 years old ($p<0.001$) (Figure 4).

1.6 Discussion

1.6.1 The magnitude of sudden cardiac death in the young

According to the ONS data, the incidence of cardiac death in the young in England and Wales is 1.8 per 100,000 per year, which corresponds to eight young lives per week. Considering the devastating impact of sudden death in the young and the potential number of life years lost our findings suggest that the number of deaths identified is sufficiently high to command attention even without the inclusion of potential misclassifications (Class B). If consideration is given to the possibility that at least 20% of deaths attributed to epilepsy or drowning may actually be caused by a primary myocardial electrical disorder, then the estimate of sudden and cardiac death in the young in England and Wales is at least 10 per week.

The incidence of SCD based on the ONS data is almost double the incidence reported by Italian and Swedish studies, which, however, are likely to have provided an underestimate in the context of cardiac screening and under-reporting, respectively.[16,29] On the contrary, our figure is half that documented in a retrospective study of sudden death in young individuals in the USA where the authors reported an incidence of 3.6 per 100,000 per year,[10,12] and almost a sixth of the incidence reported in young US military recruits.[10,15] Both studies are likely to have produced more accurate estimates compared to our study given their more robust methodology; in both studies the authors reviewed post-mortem reports and death certificates which were based on autopsy evaluation of all subjects. Our estimate is also lower compared to nationwide studies performed subsequently to our study in Ireland and Denmark, where the incidence of SCD was reported as 2.8 per 100,000 person-years.[17,18] Our lower estimate compared to the US and Irish studies may

be partly explained by the different age groups, since these studies did not include very young individuals (<15 years) in whom the incidence of SCD may be up to 10-fold lower based on our results. Therefore, it is likely that our figure derived from the ONS data is a significant underestimate of the true incidence of cardiac death in the young, given the nature of our study and the absence of systematic screening in the UK.

1.6.2 The magnitude of sudden arrhythmic death syndrome in the young

The incidence of Class A1 deaths that best correlate with SADS was 0.24 per 100 000 per year, with no significant gender or age predilection. This figure is lower compared to the majority of published studies in Europe and the US, where the incidence of SADS ranges from 0.6 to 1.2 per 100,000 per year.[10,12,15,17,18] On the other hand, this figure is significantly higher than the previous incidence of 0.10 per 100 000 per year obtained from the UK ONS statistics for 1997–1999 that used the ICD-9 classification.[7] As evident however from a prospective national coroner survey, calculations based on ONS data are likely to underestimate the true incidence of SADS.[7] The most plausible explanation for this discrepancy is that the ONS mortality statistics are derived largely from documentation on death certificates, which may under-report the true incidence of cardiac arrhythmias. Malignant cardiac arrhythmias secondary to ion channelopathies such as Brugada syndrome and long-QT syndrome may manifest as epileptiform seizures and collapse secondary to brain anoxia or drowning resulting in misclassification of genuine cases of SADS as epilepsy[33,34] or unexplained drowning.[35,36] The latter is particularly relevant since there is a well-established association between the most common subtype of long-QT syndrome, LQT1, and sudden death in swimmers.[37,38] Support to this notion is provided by our findings in the main SADS study (chapter 4), where 4 out of the 8 SADS victims who had been referred for specialist evaluation because of symptoms prior to their death, had

experienced at least one episode of generalised seizures (Table 11). All four victims had been referred to a neurologist and had undergone head imaging (CT and/or MRI) and an electroencepalogram, which in all cases were normal. In two of the victims no formal diagnosis was reached due to a single episode of seizures in the first case and nocturnal episodes, alone, in the second case. The other two victims had received diagnosis and treatment for epilepsy. Familial evaluation identified the Brugada phenotype in three families and LQT in one.

Further support that the incidence of SADS is likely to be underestimated by the ONS data is provided by the thesis studies examining the discrepancy of the interpretation of histopathological findings between general and specialist cardiac pathologist (chapter 3) and the familial evaluation of victims of SCD with autopsy findings of uncertain significance (chapter 5). Both studies indicate that the significance of post-mortem findings such as limited coronary atherosclerosis, left ventricular hypertrophy in the absence of myocardial disarray, myocardial fatty infiltration, mitral valve prolapse and myocarditis,[39] may be overestimated. Such findings may falsely be attributed as the primary cause of death, misclassifying SADS deaths under other causes of SCD. Most importantly however, overestimation of the significance of such findings may misguide familial evaluation targeting structural disorders rather than primary arrhythmogenic syndromes, with potentially devastating consequences.

1.6.3 Effect of gender and age

Consistent with prior literature reports, ischaemic or potentially ischaemic causes contributed a greater proportion of deaths in males and with increasing age, in particular after the age of 30 years. Conversely, potentially inherited cardiomyopathies such as

hypertrophic and dilated cardiomyopathies did not exhibit any gender predilection but there was a significant age trend, contributing a greater proportion of deaths in the 10 - 19 age group with a gradual decrease thereafter.

Epilepsy appeared to exhibit a female predilection, accounting for a greater proportion of deaths during adolescence. These results should however be viewed with caution given the limited data available and the multiple factors which may influence epilepsy-related mortality, as established by a prior large, prospective study in the UK.[40] Finally, in accord with previous reports, drowning-associated deaths were more prevalent among males and children aged <10 years, with a reverse trend with increasing age.[41]

1.6.4 Limitations

This epidemiological study exhibits some important limitations that warrant mention. The cause of death was ascertained from the ONS data and the authors did not examine death certificates or post-mortem reports on an individual basis in order to identify potential misclassifications. Although the ONS data do not provide information regarding the number of deceased individuals who underwent a post-mortem examination, it would be reasonable to assume that the majority of the victims were subjected to a post-mortem examination given their youth and the UK medico-legal implications.

The investigator concedes that this study relied solely on information provided to the ONS from documentation on death certificates and post-mortem reports which may not always accurately reflect the true cause of death given the ambiguities related to the diagnosis of conditions associated with sudden cardiac death in the young. This may explain the absence of conditions such as arrhythmogenic right ventricular cardiomyopathy (ARVC) as

a distinct entity, whereas minor manifestation of certain common disorders such as atherosclerosis may have been falsely attributed as the cause of death. In addition, ARVC does not have its own ICD-10 code and is classified currently as I42.8 - other cardiomyopathies. The purpose of this study, however, was to provide an estimate of the incidence of cardiac death and underlying cardiac causes in the young in order to highlight the need to establish the scale and nature of the problem.

Chapter 2: The epidemiology of sudden cardiac death and sudden arrhythmic death syndrome in athletes

(Publication attached in appendix 3)

2.1 Introduction

Most studies investigating the incidence of SCD in young athletes, defined as ≤35 years old, come from the US. One of the first studies conducted by Maron et al. utilized a unique insurance program for catastrophic injury or death, mandatory for all student athletes engaged in school sports in Minnesota.[9] As such the precise number of participants and deaths due to cardiovascular disease could be ascertained. The authors retrospectively reviewed the Minnesota State High School League records for the 12-year period, 1985/1986 to 1996/1997 inclusive, for grades 10 to 12. During that period the authors identified only 3 deaths, all classified as of cardiovascular aetiology after autopsy: 1 coronary artery anomaly, 1 myocarditis, 1 aortic valve stenosis with bicuspid aortic valve. Based on the athlete population the authors calculated the incidence of SCD as 0.46 per 100,000 per year; 95% CI: 0.09 to 1.34. All 3 deaths occurred in male athletes and the incidence for male athletes was estimated at 0.77 per 100,000 per year; 95% CI: 0.16 to 2.3.

Further studies in American high school and college athletes were performed by van Camp et al.[19] and more recently by Drezner et al.[20] and Harmon et al.[21] All three studies utilized existing athlete registries in order to estimate the total number of collegiate athletes competing over the respective study period (i.e. denominator). Depending on the study, the investigators relied on a number of different sources of reporting deaths in athletes including registries, sporting organizations, parent organizations as well as media reports. As such all studies are vulnerable to different selection biases relating to the number of deaths, which are likely to have underestimated the true incidence of SCD. The study by van Camp et al. reported an incidence of SCD of 0.75 per 100,000 per year for male athletes and 0.13 per 100,000 per year for female athletes. The study by Drezner et al was designed to assess the effectiveness of emergency response planning for sudden cardiac arrest rather than the incidence of SCD in high school athletes. Based on their findings however, the study reported an incidence of sudden cardiac arrest in high school student athletes of 4.4 per 100,000 per year. Although this estimate may have been influenced by responder bias, it is consistent with recent findings from a prospective, population-based study of paediatric out-of-hospital cardiac arrest in which the incidence of cardiac arrest caused by cardiovascular disease in adolescents 14 to 24 years was 3.75 per 100 000 person-years.[42] Finally, the study by Harmon et al. reported an incidence of SCD of 2.3 per 100,000 per year, highlighting a considerable gender (male versus female athletes - 2.3 to 1), ethnicity (black athletes versus white athletes - 3.3 to 1) and sport (basketball versus cross-country - 3.7 to 1) predilection.

In a second study by Maron et al.[14] the authors attempted to calculate the incidence of SCD in US competitive athletes, based on the data from a US National registry of sudden death in athletes and the estimated number of participants in all competitive sports ≤39

years old in the US during the same time period. The authors identified 1866 deaths over a 27-year period of which 1049 were classified as cardiovascular. The authors were able to confirm the cause of death by reviewing the autopsy report in only 690 (66%) cases of potentially cardiovascular deaths. Cardiomyopathies accounted for the majority of deaths (51%), followed by coronary artery pathology and predominantly coronary artery anomalies (21%) and myocarditis (6%). In contrast to the general population studies deaths attributed to SADS accounted for less than 4%. The incidence of sudden deaths in these athletes was reported as 0.61 per 100 000 person-years. Although the attempt to database SCDs in young athletes is admirable, the methodology employed to collect the data, such as internet searches and media accounts, makes the results prone to numerous selection biases, which are likely to lead to inaccurate conclusions. Essentially, there is no reliable numerator or denominator, subjects were subjected to different post-mortem investigations and there is also an issue of reliability of post-mortem results since they were performed by numerous pathologists across the US.

The only large European study addressing systematically the incidence of SCD in young athletes is the one by Corrado et al.[22] The study is unique in that a well-defined population of athletes and non-athletes in the Veneto region of Italy was prospectively studied for 25 years. Most importantly however, and in contrast to all other studies, because of the highly organized referral network, all deaths in young (12-35 years) individuals considered to be of cardiac cause were referred for post-mortem evaluation to a single centre and underwent detailed histopathological evaluation of the heart by a small number of expert cardiac pathologists. Moreover, the unique pre-participation screening program which is enforced by law in Italy ensures that athletes participating in formal competition are subjected to annual medical reviews. Based on the results of this study the incidence of SCD in young athletes was estimated at 4.19 per 100,000 per year prior to the initiation of

the pre-participation screening program. The incidence of SCD in young non-athletes was around 1.0 per 100,000 per year with no significant fluctuations over the 25-year period. In the Veneto cohort, rhythm and conduction abnormalities, which could be considered to represent SADS deaths, accounted for 39% of cardiac pathology.

2.2 Aim

The incidence of SADS in athletic populations in the UK has not been studied and in a similar manner to young individuals, in the absence of a systematic national registry documenting sudden cardiac deaths in athletes, the true impact of such fatalities remains speculative. A considerable proportion of SCDs in athletes are secondary to previously quiescent cardiac diseases, galvanizing discussions relating to primary and secondary prevention strategies to avert such catastrophes, particularly when one considers that athletes are perceived as the healthiest segment of society. Establishing the magnitude and pathogenesis of sudden cardiac death in young athletes is fundamental to facilitate any debate about local provisions for a potential cardiovascular screening programme.[28-30] Finally, establishing the impact of SADS may also inform the debate as to the most appropriate screening protocol since the 12-lead ECG is the principal diagnostic tool for identifying all primary arrhythmogenic syndromes.

To address the scientific gap relating to the magnitude of SADS in athletic individuals in the UK we conducted a study in collaboration with the Cardiac Risk in the Young (CRY) centre for cardiac pathology at Royal Brompton Hospital. The authors performed a retrospective analysis of 118 hearts referred to the cardiac pathology centre, of people who participated in regular sport activities and experienced sudden death, between 1996

and 2008. The contribution of individual underlying cardiac pathologies and in particular the finding of a normal heart (SADS) was reported.[43]

2.3 Personal contribution

The author reviewed data relevant to the project and assisted with analysis of data and drafting of the published manuscript. The author was not involved with the post-mortem evaluation or toxicology screen of any of the subjects or the initial raw data collection that was performed by our cardiac pathology team (Dr Sofia De Noronha, Prof Mary N Sheppard).

2.4 Methods

Between January 1996 and July 2008, 118 cases of sudden death in people participating in regular sport activities, defined as ≥ 2 hours per week, were referred to the Cardiac Risk in the Young (CRY) Centre for Cardiac Pathology at the Royal Brompton Hospital for further evaluation, by coroners and pathologists throughout the UK. Subjects were divided into two groups based upon their age at death: (a) ≤35 years and (b) >35 years. Data on age, sex, circumstances of death, sporting discipline, antecedent cardiac symptoms, past medical history and a family history of cardiac disease (when available) of the deceased were obtained from the referring pathologist or coroner.

2.4.1 Post-mortem evaluation

The hearts were referred to the Cardiac Risk in the Young (CRY) Centre for Cardiac Pathology at the Royal Brompton Hospital with the consent of the coroner and the family of the deceased. A specific protocol was established for handling all SCD referrals, summarised in Figure 5.

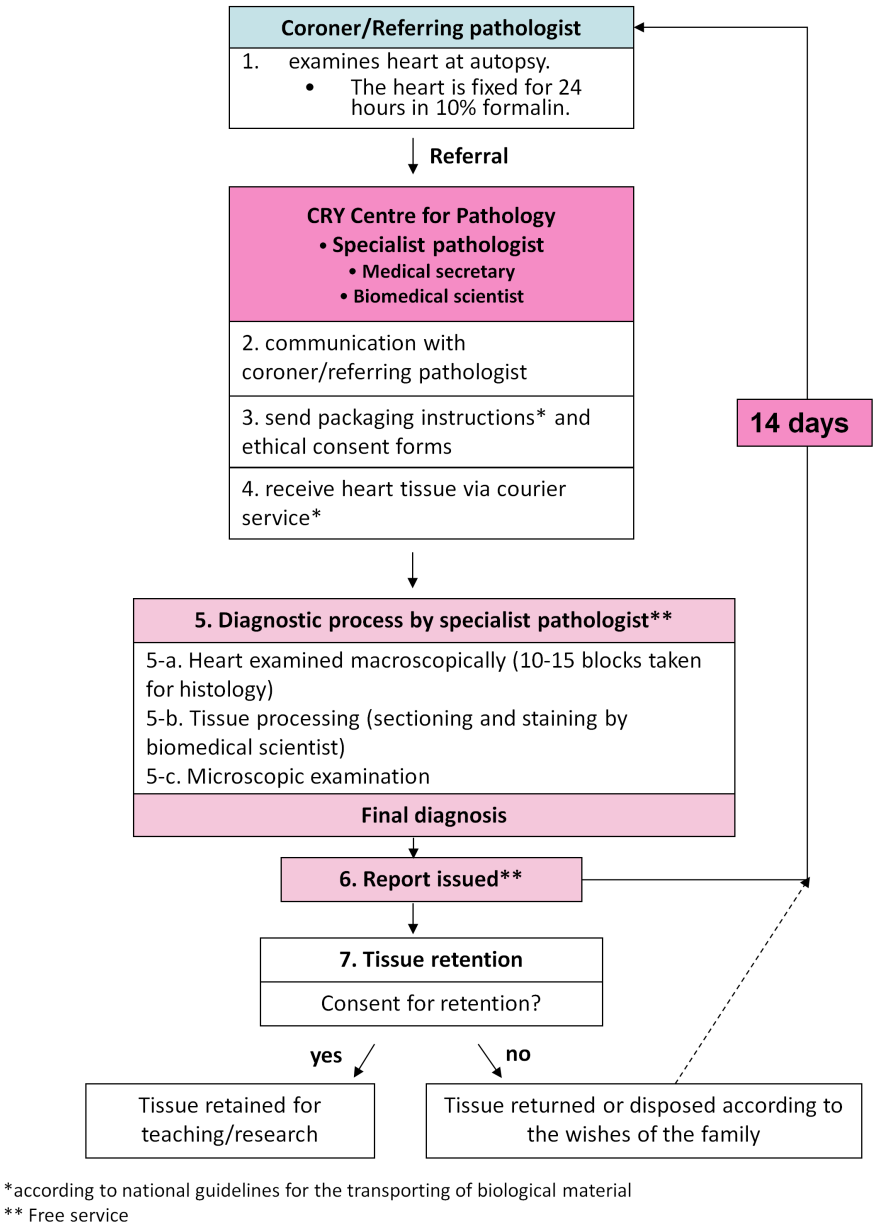


Figure 5: Flow chart depicting the cardiac referral procedure at the Cardiac Risk in the Young (CRY) centre for cardiac pathology.

Pathological analysis

Pathological analysis of all hearts was performed by a single cardiac pathologist (MNS) with the consent of the coroner and family of the deceased. The heart weight was recorded and measurements of the left and right ventricular wall thickness and internal cavity dimensions were made at mid-ventricular level excluding papillary muscle and fat. The extramural coronary arteries were studied macroscopically in the intact heart by making multiple cross sections of the vessels (3–5mm apart). Multiple (10 to 20) tissue sections were routinely taken according to agreed national and international criteria[44] and included: the right ventricular outflow tract; a right lateral cut containing right atrium, posterior leaflet of the tricuspid valve and lateral right ventricle; a left lateral cut containing left atrium, mitral valve and lateral left ventricle; circumferential right and left ventricle samples; the anterior and posterior septum; the three major coronary arteries; the ascending aorta; and the conduction system. Extra tissue sections were taken to confirm pathology when this was detected macroscopically or microscopically. Sections were fixed in formalin, embedded in paraffin and stained with haematoxylin and eosin stain or elastic Van Gieson to highlight myocardial fibrosis. Table 5 summarises the macroscopic and histological criteria for specific cardiac diseases, as defined by MNS, based on established literature. Results were reported in four broad categories: (a) cardiomyopathies; (b) coronary artery pathology; (c) morphologically normal heart and (d) other cardiac pathology, including myocardial inflammation and valvular heart disease.

All patients included in the studies underwent a toxicology screen as part of the coroner's mandate since all deaths were sudden and unexpected.

Table 5: Macroscopic and microscopic definitions of cardiac pathology		
Disorder	**Macroscopic appearance**	**Microscopic appearance**
Cardiomyopathy		
HCM	Left ventricular wall thickness ≥15 mm and or heart weight ≥500g in males or ≥400g in females.	Myocyte hypertrophy, significant disarray (≥2 LV sections) and interstitial and/or replacement fibrosis
Idiopathic LVH (Hypertensive heart disease if clinical history of hypertension)	Left ventricular wall thickness ≥15 mm and or heart weight ≥500g in males or ≥400g in females.	Myocyte hypertrophy with or without fibrosis. No myocyte disarray.*
ARVC	Right ventricular dilatation and thinning with fatty replacement and fibrosis. Epicardial fat and fibrosis in the outer left ventricle.	Fibro-fatty replacement, mainly epicardial extending to replace the full thickness of the right ventricular wall. Left ventricular involvement occurs with subepicardial fibrous tissue and fat.
Obesity cardiomyopathy	Left ventricular wall thickness >15mm and/or heart weight ≥500g in males or ≥400g in females with a BMI ≥30Kg/m^2	Myocyte hypertrophy, nuclear enlargement, with or without fibrosis. No myocyte disarray.*
DCM	Enlarged heart with heart weight ≥500g in males or ≥400g in females. Dilated ventricular chamber (>40mm), circumferentially thin walled (<10mm) with mid-myocardial fibrosis.	Replacement and interstitial fibrosis throughout the left ventricle. Degenerative changes in the myocytes. Myocyte size varies from atrophied to hypertrophied.
Idiopathic fibrosis	Normal left ventricular thickness ≤15 mm	Focal and diffuse interstitial and/or replacement fibrosis in the ventricular wall
Coronary artery pathology		
Atherosclerosis	Atherosclerosis with narrowing >75%	Rupture/thrombosis of coronary artery and/or acute/chronic infarction in the LV
Anomalous coronary artery	Anomalous origin of the coronary artery, coronary artery atresia, stenosis	Fibrosis/acute/chronic infarction in the left ventricle
Coronary artery spasm	Circumferential subendocardial haemorrhagic infarction with normal coronaries	Acute infarction in the LV

Table 5: Macroscopic and microscopic definitions of cardiac pathology		
Coronary dissection	Tear in the coronary artery media	Infarction in the territory of the artery
Coronary artery bridging	Coronary artery bridging was considered pathologically significant when the left anterior descending coronary artery had a long (15-30mm) and deep (≥3mm) intra-myocardial course.	Ischaemic damage in the antero-septal left ventricle.
Morphologically normal heart		
Normal heart	Normal	Normal
Other cardiac pathology		
Myocarditis	Normal or dilated ventricle	Inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes, in the absence of coronary artery disease. >14 leucocytes/mm^2 with the presence of T- lymphocytes >7 cells/mm^2.
Mitral valve rupture	Mitral valve papillary muscle or chordae tendineae rupture with marked ballooning of both leaflets above the atrioventricular junction	Myxoid change in the valve with associated fibrosis.
Floppy mitral valve	Mitral valve thickened with prolapse of both cusps into the left atrium.	Myxoid change in the valve
Aortic dissection or rupture	Tear in the proximal aorta above the aortic ro haemopericardium.	Focal to widespread cystic medial change in the aorta.
Bicuspid valve	Two leaflets associated with nodular calcification with significant aortic orifice stenosis.	Left ventricular hypertrophy with or without fibrosis
Sickle cell crisis	Normal	Sickle cell in intramural coronary vessels with micro-infarcts in the myocardium

* isolated myocyte disarray confined to the anteroseptal and posteroseptal junctions should be considered normal

ARVC: Arrhythmogenic right ventricular cardiomyopathy; DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy; LVH: Left ventricular hypertrophy

Ethical approval

Trust gebnerated approval was obtained from the Brompton, Harefield and National Heart and Lung Institute.

Statistical analysis

Data interpretation and analyses were performed using Stata version 10.1 (Statacorp, Texas USA). Means and standard deviations (SD) were calculated for continuous variables. Group differences are examined using t-test where appropriate. A value $p<0.05$ was considered statistically significant throughout.

2.5 Results

2.5.1 Demographics

Of the 118 cases of SCD, the majority were amateur sports participants (n=107, 91%) and included seven subjects who had participated in 2–23 marathons. The remaining 11 cases were seven athletes at a professional or semi-professional level (soccer n=6, cycling n=1) and four who participated in intensive physical training in the armed forces.

The subjects were predominantly male (n=113; 96%). One hundred and thirteen athletes (96%) were white and five were black (African/Caribbean in origin). The mean (SD) age of SCD in this series was 27.9±12.5 years (range 7–59). Seventy-five per cent of all deaths were in subjects aged ≤35 years and almost one-third were in child or adolescent athletes (<18 years). The greatest number of deaths (n=20) occurred in the 16–20 year age group

(Figure 6). With the exception of one case, all female athletes who died were in the younger age group.

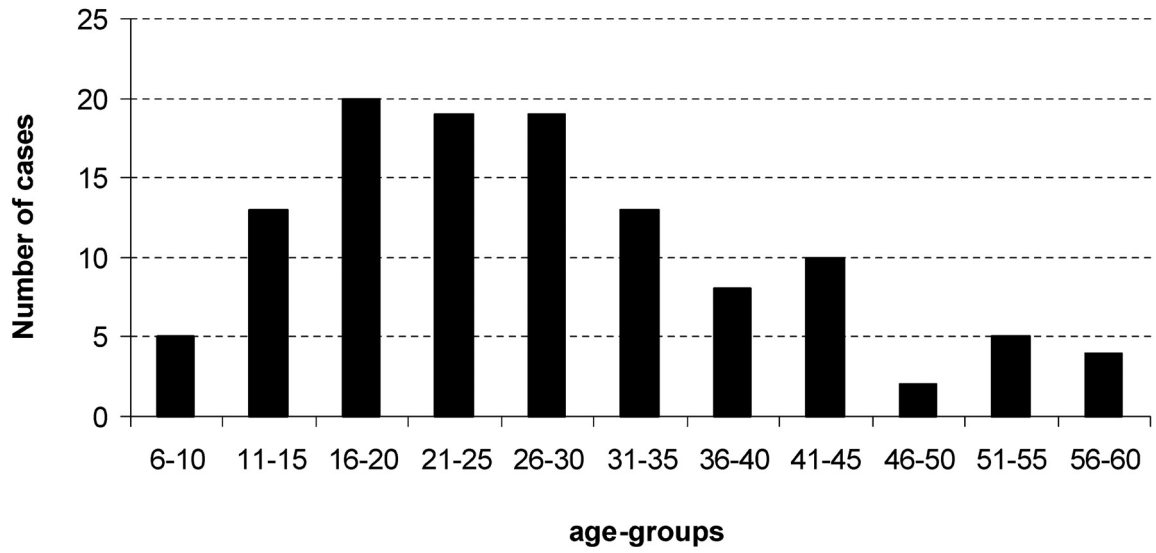


Figure 6: Bar chart showing the number of sudden deaths in athletes in relation to age in 118 deaths in sportsmen referred to a tertiary centre in the UK over a 12-year period.

The vast majority of SCDs (81%) occurred during (66%) or immediately after (15%) exercise. In relation to sporting discipline, most deaths occurred in soccer, followed by running and rugby (Table 6).

Table 6: Demographic characteristics of the athletic cohort	
Characteristics	**Values**
Number of subjects	118
Male	113 (96%)
Age (years), mean±SD, {range}	27.9±12.5, {7–59}
Number of subjects ⩽35 years	89 (75%)
Sport discipline	
Soccer	44 (37%)
Running	24 (20%)
Rugby	11 (9%)
Cycling	8 (7%)
Swimming	5 (4%)
Weight lifting	3 (3%)
Golf	3 (3%)
Other (⩽2 subjects/discipline)	20 (17%)

2.5.2 Antecedent symptoms, past medical history and family history of cardiac Disease

Of the 118 cases, 21 (18%) had experienced one or more antecedent cardiovascular symptoms which could be attributed to underlying cardiac disease (Table 7). In 20 subjects (17%) there was a family history of premature cardiovascular disease (≤50 years old), with IHD in the majority of cases (69%). In 8 (7%) cases there was a family history of premature SCD in a first-degree relative. Twenty-five (21%) of the subjects had relevant previous medical history; 7 had a history of confirmed cardiac disease, 9 were clinically suspected to have underlying cardiac pathology, 5 had risk factors for coronary artery disease and 4 had had at least one epileptic seizure (Table 7). Ten athletes (8%) had a previous diagnosis of asthma. As far as the authors can ascertain, none of the subjects had been subject to pre-participation cardiovascular evaluation to identify disorders capable of causing SCD.

Table 7: Antecedent symptoms, family history and relevant cardiac history of the 118 subjects *			
Patient information	**Number of cases n=118**	**Age in years**	
		≤ 35 n=89	**> 35 n=29**
Family history premature SCD (≤50 years old)	20 (17%)	15 (17%)	5 (17%)
Symptoms *	21 (18%)	16 (18%)	5 (17%)
Syncope/dizziness	10 (9%)	9 (10%)	1 (3%)
Shortness of breath	7 (6%)	5 (6%)	2 (7%)
Palpitations	6 (5%)	6 (7%)	0
Chest pain	4 (3%)	3 (3%)	1 (3%)
Relevant past medical history	25 (21%)	16 (18%)	9 (31%)
Clinical suspicion of heart disease #	12 (10%)	9 (10%)	3 (10%)
Seizures	4 (3%)	4 (5%)	0
Diabetes	3 (3%)	1 (1%)	2 (7%)
Hypercholesterolaemia	4 (3%)	0	4 (14%)
Congenital heart disease	3 (3%)	3 (3%)	0
Previous cardiac arrest	2 (2%)	1 (1%)	1 (3%)
Atrial fibrillation	1 (1%)	0	1 (3%)
Viral myocarditis	1 (1%)	1 (1%)	0

* symptoms/findings do not add up since some subjects had more than one symptom/finding.

abnormal ECG; n=4, abnormal echo; n=2, heart murmur; n=3, possible arrhythmia; n=3.

2.5.3 Causes of sudden cardiac death

Abnormal cardiac pathology (macroscopic and/or microscopic) was identified in 91 (77%) of all cases. The remaining subjects had a morphologically normal heart (Figure 7 and Table 8).

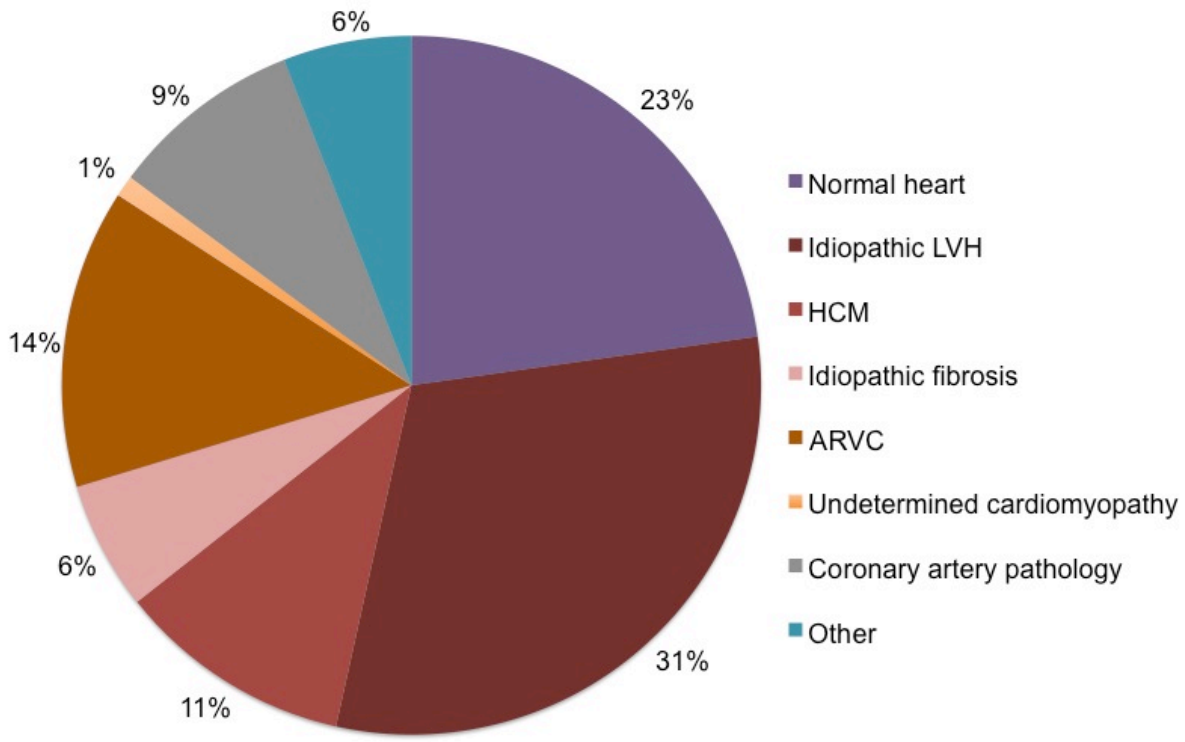


Figure 7: Pie chart showing the causes of sudden cardiac death in 118 sports deaths referred to a tertiary cardiac centre in the UK over 12 years.

ARVC, arrhythmogenic right ventricular cardiomyopathy; HCM, hypertrophic cardiomyopathy; LVH, left ventricular hypertrophy. Percentages do not add up to 100% because of rounding.

Toxicology screen

All but two subjects had a normal toxicology screen. Both were body builders and had blood traces of anabolic steroids.

Cardiomyopathy

Deaths attributed to a primary myocardial disorder (cardiomyopathy) were identified in 73 (62%) of all athletes. Left ventricular hypertrophy (LVH) was the most commonly identified abnormality on macroscopic examination and was detected in 49 (42%) athletes raising the possibility of HCM. However, only 13/49 (27%) athletes with LVH exhibited associated myocyte disarray, the historically regarded histological hallmark of HCM. The remaining cases of LVH (n=36; 31%) were associated with histological evidence of either isolated myocyte hypertrophy (n=27) or myocyte hypertrophy and fibrosis (n=9). The authors classified these cases under the term ‘‘idiopathic LVH’’. One case of HCM and another of idiopathic LVH were associated with the presence of anabolic steroid traces on toxicology screen.

Athletes with HCM were predominantly in the younger age group (≤35 years) with a mean age of 24.6±7.1 years (range 11–43) and all but one case were male. Similarly, 71% of all victims with idiopathic LVH were in the younger age group with a mean age of 32.7±12.6 years (range 9–59) and all were male. Of the five Afro-Caribbean subjects, four had evidence of idiopathic LVH.

Arrhythmogenic right ventricular cardiomyopathy was the second most common diagnosis and was identified in 16 (14%) members of our cohort. There was evidence of biventricular involvement in 50% of cases. In contrast with cases of idiopathic LVH and HCM, deaths were equally distributed between the older and younger age group with a mean age of 35.9±12.2 years (range 16–57).

Idiopathic fibrosis without LVH occurred in seven cases with all but one in the younger age group with a mean age 28.4±8.5 years (range 17–43). Finally, one case exhibited features of both ARVC and HCM. There was insufficient tissue sampling to provide a definite diagnosis and the case was classified as an undetermined cardiomyopathy.

Table 8: Cause of sudden cardiac death according to histopathological findings

Diagnoses	**⩽35**	**>35**	**Total**
Cardiomyopathy	*49*	*24*	*73*
Idiopathic left ventricular hypertrophy	19	8	27
Idiopathic left ventricular hypertrophy with fibrosis	3	6	9
Arrhythmogenic right ventricular cardiomyopathy	9	7	16
Hypertrophic cardiomyopathy	11	2	13
Idiopathic fibrosis	6	1	7
Undetermined cardiomyopathy	1	0	1
Morphologically normal heart	*26*	*1*	*27*
Coronary artery pathology	*7*	*4*	*11*
Anomalous coronary artery	6	0	6
Atherosclerosis	0	3	3
Coronary “spasm”	1	0	1
Coronary dissection	0	1	1
Other cardiac pathology	*7*	*0*	*7*
Myocarditis	3	0	3
Floppy mitral valve	2	0	2
Complex congenital heart disease	1	0	1
Sickle cell crisis	1	0	1

Coronary artery pathology

Coronary artery pathology was identified in 11 (9%) subjects. The main pathology was a congenital anomaly of the coronary arteries which was seen in six subjects all of whom were male and ≤35 years old (mean age 15.8±6.2 years; range 7–25). Both coronary arteries arose from the same coronary ostium in four cases; two cases had the left coronary artery arising from the right sinus and two cases had the right coronary artery

arising from the left sinus. Of the remaining two cases, one had atresia and hypoplasia of the left coronary artery and the other stenosis of the ostium and a shelf-like slit of the left coronary artery. Coronary atherosclerosis was the cause of death in only three athletes who were all aged >35 years (mean age 49.7±4.0 years; range 45–52). A single case of coronary artery spasm was detected in a 17-year-old man, as well as one case of spontaneous dissection of the left anterior descending artery in a 38-year-old man.

Other cardiac pathology

Lymphocyte myocarditis was the predominant finding in three cases. Two subjects had valvular disease which included floppy mitral valve and associated myocardial fibrosis. There was a single subject with corrected, complex congenital heart disease of univentricular circulation and Fontan circulation who exhibited biventricular hypertrophy with no other associated anomalies. Finally, one subject had evidence of an acute sickle cell crisis with sickling within the coronary arteries and associated acute ischaemia.

Morphologically normal heart

In almost a quarter of our cohort (23%) the post mortem disclosed no cardiac abnormality which could account for the cause of death, despite detailed macroscopic and microscopic examination. The majority of these cases (96%) were in the younger age group. The mean age of victims with a morphologically normal heart was 18±6.1 years (range 8–42) which was significantly lower than that of those dying with identifiable cardiac pathology ($p<0.001$). Interestingly, three of the five swimmers who died had a morphologically normal heart.

Female athletes

Of the five female athletes in the series, two had a morphologically normal heart, one had HCM, one exhibited idiopathic fibrosis and one coronary atherosclerosis.

2.6 Discussion

The cardiovascular benefits of regular physical activity are well established[45] and only a small proportion of athletes with unsuspected cardiac pathology are at increased risk of exercise-related SCD.[9,11,13,14,22,46] The majority of data examining the aetiology of deaths in athletes originates from the USA (690 cases of primary cardiovascular death),[14] although a number of smaller studies exist in European countries, including France (80 cases)[47] Spain (61 cases),[48] Italy (55 cases),[22] and Ireland (51 cases).[49] Data in the UK are scarce and limited to a small group of older sport participants. This study of 118 SCDs in athletic individuals is the largest reported series in the UK.

2.6.1 Sport and gender predilection

Consistent with large American[14] and Italian series[22,50] of SCD in athletes, just over 80% of deaths occurred during or immediately after exercise, indicating that the interplay of physical, metabolic and endocrine stresses of exercise on the heart is an important trigger for fatal ventricular arrhythmias. Soccer and running were the sports most commonly associated with SCD and this is in agreement with most other studies.[9,13,14,22,30,46,50] This sport bias most certainly represents the high participation rates in these sporting disciplines in most Western European countries. In concurrence with previous studies, the great majority (96%) of SCD subjects in our cohort were male.[9,14,22,46-52] Based on our ONS

data male gender appears to confer some degree of increased risk (2.5 to 1) of SCD outside the context of sports. However, within the context of this study, male predominance (23 to 1) may be largely attributed to the lower participation rate of women in sport generally and specifically in sports popular with male subjects, such as soccer, which is the predominant sport in our study.

2.6.2 Morphologically normal heart

Despite detailed macroscopic and histological examination by an expert cardiac pathologist, in a quarter of the cohort (23%) no structural cardiac abnormality was identified, implying a SADS death. Previous studies have reported significantly lower rates, as low as 1%,[14,46,51] with only a Spanish series reporting a figure comparable to our study (16.3%).[48] Although selection bias has certainly contributed to the high prevalence of SCD with a morphologically normal heart, this study highlights the importance of establishing such a diagnosis since primary arrhythmogenic syndromes can be identified in relatives and appropriate treatment instigated to avoid further tragedies.[53] Of interest, three children (aged 7–15 years) with morphologically normal hearts died during swimming. This sport has been particularly associated with deaths in LQTS[54,55] and CPVT.[36]

2.6.3 Cardiomyopathy

Consistent with previous studies in the USA[9,14] and Italy,[22,50] our results indicate that cardiomyopathies are the most prevalent underlying pathology in SCD related to athletic activity. In this series, however, LVH without myocyte disarray, was the predominant finding (31%), compared with HCM and ARVC in the USA and Italy, respectively. Idiopathic LVH is becoming increasingly recognised and although it has been reported in

previous studies, this is the first series in which it predominates. Its exact significance at this stage is unclear and was the subject of investigation in our study of post-mortem findings of uncertain significance (study V). In the context of athletic individuals it may represent an acquired pathological variant of the physiological LVH exhibited as part of the ‘‘athlete’s heart’’ in certain genetically predisposed backgrounds.[56] The finding of idiopathic LVH in four out of five Afro-Caribbean cases of SCD may be relevant in this regard since a recent study by our group in highly trained black athletes showed that 3% exhibit substantial LVH (>15 mm) and it is plausible that in such athletes, marked LVH predisposes to exercise-related fatal ventricular arrhythmias.[57] LVH has also been associated with the use of anabolic steroids.[58] In our study traces of anabolic steroids were identified at post mortem in two body builders; one was diagnosed with idiopathic LVH and the other with HCM.

Idiopathic myocardial fibrosis with or without LVH, featured in 14% of this cohort in contrast to significantly lower rates in previous studies (2%–3%).[47-49] The aetiology and importance of cardiac fibrosis also remains unclear; however, transient myocardial damage has been detected in athletes in the post-race setting and has been associated with transient diastolic and systolic dysfunction.[59] Possibly, in some athletes prolonged arduous physical activity may result in myocardial necrosis and subsequent fibrosis. This pathology may represent an acquired, exercise-related cardiomyopathy and/or genetic predisposition leading to a fatal arrhythmia. This concern was raised in a recent case report from our group of a marathon runner who died during a race with marked LVH and myocardial fibrosis.[60] It is also possible that at least some of these cases may be due to the recently recognised familial arrhythmogenic left ventricular cardiomyopathy.[61]

ARVC was the second most common cardiomyopathy and accounted for 14% of all sudden deaths in our series. Our figures were significantly higher than those reported in the US series[9,14] and not dissimilar from the reports from the Veneto region of Italy[22,50,51,62] and other European countries.[16,47,48,63,64] These observations suggest that there may be a higher genetic cluster of ARVC in Europe or, owing to the Venetian experience, a greater awareness of this disorder amongst pathologists in Europe. Moreover, recent studies have demonstrated that intense endurance exercise causes acute dysfunction of the RV.[65] Although short-term recovery appears complete, chronic structural changes and reduced RV function are evident in some of the most practiced athletes, leading to the hypothesis that an ARVC-like phenotype may be acquired through intense exercise.[66]

Of note, we did not observe deaths from dilated cardiomyopathy in our cohort, which contrasts with series from other countries where the range was 2–11%.[46-48,51]

Coronary artery pathology

Coronary artery pathology was less prevalent in this study than in the US and Italian experience. Sudden cardiac death secondary to anomalous coronary arteries was confined to the younger age group (median age of 17 years) in this series, a trend supported by previous reports.[9,14,22,47,48,50] Atherosclerotic coronary artery disease was seen in a much smaller number of individuals and was confined to the older (>35 years) age group as observed in previous sports deaths series.[49,52,67]

2.6.4 Clinical implications related to pre-participation screening

The absence of antecedent cardiac symptoms and/or a family history of cardiac disease and/or SCD in almost 80% of cases confirm prior observations that most cardiovascular disorders responsible for SCD in the athlete are clinically silent and unlikely to be discovered from spontaneous symptoms. Cardiovascular screening with ECG testing has been associated with a significantly higher diagnostic yield than reliance on history alone, although concerns remain relating to the high false positive yield and the need for unnecessary investigations and potential false disqualification from sport.[11,27,68-70] This study indicates that based on the high prevalence of cardiomyopathies and the relatively low occurrence of coronary artery pathology in our subjects, pre-participation cardiovascular screening using a 12-lead ECG may have raised suspicion of an underlying cardiac abnormality in a significant proportion of SCD victims. This argument is further reinforced by the high prevalence of SCDs in people with a morphologically normal heart, of which a significant proportion may be attributed to inherited ion channelopathies, which might be potentially detected by the 12-lead ECG.

We concede that almost one-third of the cases in this series exhibited idiopathic LVH and it may be argued that these cases may not have been identified with ECG. It is unclear at this stage whether idiopathic LVH represents a spectrum of the HCM phenotype, an exaggeration of the physiological response[56] resulting in pathological LVH or whether it is indeed an innocent bystander (genuine physiological LVH) in a person who may have succumbed to a fatal ventricular disorder owing to an undetectable ion channel disorder or congenital accessory pathway. However, it is well established that over 90% of people with HCM have an abnormal ECG,[71] and athletic individuals with LVH on

echocardiography are more likely to exhibit repolarisation anomalies, and in particular T-wave inversions, in their ECG.[72]

The predominance of deaths due to idiopathic LVH and idiopathic myocardial fibrosis does raise the possibility that some deaths in sport may be secondary to acquired myocardial disorders resulting from the long-term effects of intensive exercise, warranting several cardiac assessments throughout the athlete's career.

The Italian screening programme in athletes has been successful in identifying and preventing deaths predominantly from the cardiomyopathies through subsequent disqualification of the affected subject from sporting activities of moderate to high intensity to minimise the risk of SCD. In this regard it is prudent to highlight that in this study almost one-fifth of all SCDs in athletes occurred at rest, suggesting that the identification of cardiovascular diseases and subsequent disqualification from sport will not necessarily prevent deaths in all athletes harbouring potentially fatal cardiac disorders.

2.6.5 Limitations

We concede that conclusions drawn from this study do not necessarily apply to the whole of the UK because of a significant selection bias. The CRY Centre for Cardiac Pathology at the Royal Brompton Hospital is an internationally recognised cardiac pathology centre in the UK, where the hearts of many young athletes are commonly referred, especially when the findings are ambiguous and no clear cause of death can be established by the local pathologist. It is therefore highly probable that cardiac anomalies such as coronary artery atherosclerosis, which can be easily identified, and cardiomyopathies such as HCM, which have been well characterised, are under-represented in this cohort. Similarly the

prevalence of less well-defined entities such as idiopathic left ventricular hypertrophy and a morphologically normal heart are likely to represent an overestimate.

Diagnostic criteria reported in table 5 are based on National and International recommendations but also on the experience of our cardiac pathologist (MNS).[44,73] As a result certain definitions, such as the differentiation of HCM from idiopathic LVH, may be considered arbitrary rather than evidence based. In addition, the post-mortem diagnosis of a potential cause of SCD in all athletic individuals was based on the analysis and interpretation of the autopsy findings by a single pathologist (MNS), albeit an acknowledged international expert. As such we were unable to provide inter-observer variability. Having an additional pathologist to corroborate the cardiac pathological interpretation would be desirable, but single centres cannot justify more than one cardiac pathologist. There are also few cardiac pathology specialists in the UK, posing a limitation on double reporting.

Chapter 3: Post-mortem evaluation of victims of sudden cardiac death - Impact of cardiac pathology

(Publication attached in appendix 3)

3.1 Introduction

Post-mortem examination is a critical first diagnostic step required to guide clinical evaluation of surviving relatives of victims of SCD. In the UK the pathologist's report is commonly the sole determinant as to whether relatives are referred for familial evaluation for potentially inherited cardiac conditions and more specifically whether subsequent investigations are directed towards structural disorders or primary arrhythmogenic

syndromes. Best practice guidelines set by the Royal College of Pathologists and the Association for European Cardiovascular Pathology recommend referral of the whole heart to specialist centres with high volume of cardiac autopsies and established expertise.[44,73] According to both governing bodies all potential sudden cardiac deaths should be subjected to a comprehensive macroscopic and histological evaluation as well as toxicology screen. The pathologist should address the following subjects; 1. whether the death is attributable to a cardiac disease or to other causes of sudden death; 2. the nature of the cardiac disease, and whether the mechanism was arrhythmic or mechanical; 3. whether the cardiac condition causing sudden death may be inherited, requiring screening and counselling of the next of kin; 4. the possibility of toxic or illicit drug abuse and other unnatural deaths.

Despite the guidelines however, there is widespread variation in Europe as to the rate of autopsies for SCD even within the same country.[74] An observational study by Winkel et al. surveyed the rate of autopsies performed in different counties in Denmark after a sudden death in individuals aged between 1-35 years. The authors identified a significant difference in the rate of autopsies performed ranging from 60% to 88%. Autopsies were more often conducted in urban areas compared to rural areas and in East Denmark compared to West Denmark. The authors concluded that despite operating under the same set of laws, there were significant regional differences in forensic investigations of young persons suffering a sudden unexpected death and called for a revision of the way these deaths are handled.

In the UK all sudden unexpected deaths, particularly in young individuals, have an autopsy carried out by a coroner's pathologist. Most British pathologists, however, have limited experience with conditions predisposing to young SCD, with few resources and time

constraints. Moreover, the introduction of the Human Tissue Act in 2004 has limited preservation of tissue at autopsy.[75,76] All these factors translate to either the absence or limited histopathological examination in cases of SCD, which remains unsatisfactory and may lead to erroneous diagnoses.[3,7,77-79] In 2006 the National Confidential Enquiry into Patient Outcome and Death (NCEPOD) audit of coronial autopsy reports in England, Wales and Northern Ireland was critical of autopsy practice.[80] The audit reported that a high proportion of coronial autopsies did not comply with the guidance issued by the Royal College of Pathologists. A further on-line survey of pathologists in the UK aimed to provide an overview of current autopsy practice.[77] Of the 1213 pathologists invited, only 406 completed the survey. Results confirmed considerable pressures of time and resources and limitations on examination and sampling. The survey concluded that the circumstances under which coronial autopsies are conducted in many parts of the UK make it difficult or impossible to comply with the Royal College of Pathology guidelines, which set higher standards than what can be achieved in routine practice.

A recent study published by Wilms et al.[78] evaluated the reporting of autopsies performed in the investigation of sudden unexplained death in the young in New Zealand. Similarly to the UK, New Zealand has established guidelines, introduced in May 2008, that closely reflect those of the Royal College of Pathologists. The study was based on the fact that all cases of sudden and unexpected deaths in 0–40 year olds are referred to the cardiac inherited disease group, which the authors reviewed. Two periods were reviewed corresponding to a period before and after the new guidelines on autopsy practice, in order to assess the impact of such guidelines. Data were examined in four sections: 1. details of presentation and previous history; 2. data gathered at the time of the post-mortem; 3. results of histological tests and toxicology; and 4. analysis of the coronary arteries. One hundred and ten deaths were included in the study. Relating to the post-

mortem evaluation of the victims, over 95% listed heart weight, valvular examinations, pulmonary and some myocardial histology. Less than 50%, however, commented on septal, left ventricular and right ventricular wall thickness. Less than 50% mentioned the site of histology samples, or gave specific description of left ventricular or right ventricular histology or conduction system. Toxicology was not mentioned in a third of cases. Histology of coronary arteries was described in less than a fifth of victims (18%). After the publication of the guidelines some improvement of reporting was noted, with a higher reporting rates of the number and location of histology samples (from 0 to 47%) and higher reporting rates of coronary artery histology (from 6% to 50%). The authors concluded that most autopsy reports fall short of best practice guidelines and need to be more consistent and in particular, include significant negative findings.

3.2 Aim

To investigate concerns over autopsies performed by general pathologists and the potential for erroneous diagnosis by comparing diagnostic discrepancies between general pathologists and a specialist cardiac pathologist.

3.3 Personal contribution

The author reviewed data relevant to the project and assisted with analysis of data and drafting of the published manuscript. The author was not involved with the post-mortem evaluation or toxicology screen of any of the subjects or the initial raw data collection that was performed by our cardiac pathology team (Dr Sofia De Noronha, Prof Mary N Sheppard).

3.4 Methods

The charity Cardiac Risk in the Young (CRY) launched the Centre for Cardiac Pathology in the National Lung and Heart Institute (Imperial College) in March 2007 to address the need for detailed, high-quality histopathological evaluation of cases of SCD from potentially inherited conditions, by a cardiac pathologist, at no cost to the family, coroner or health service. The authors prospectively reviewed a sample of 200 consecutive cases of SCD referred to the CRY centre of cardiac pathology between March 2007 and December 2009. Inclusion criteria were as follows: 1. referral by a coronial pathologist of a witnessed instantaneous death or a suspected sudden death, 2. the individual was seen alive and well up to 24 hours prior to death, 3. non-cardiac causes excluded at initial autopsy, 4. Negative toxicology screen, 5. the referring pathologist had performed pathological evaluation of the heart and provided a potential cardiac cause accounting for the sudden death. All ages were included. One hundred and fifty eight cases fulfilled all criteria and were included in the study. The 158 autopsies of SCD victims were performed by local pathologists in 45 different counties within the UK. Data on age, sex, location/circumstances of death of the deceased were obtained from the referring pathologist or coroner.

The methodology relating to specimen referral process (figure 5) and pathological analysis (table 5) is identical to chapter 2.

Ethical approval

Trust generated approval was obtained from the Brompton, Harefield and National Heart and Lung Institute.

Statistical analysis

Data interpretation and analyses were performed using Stata version 10.1 (Statacorp, Texas USA). Means and standard deviations (SD) or median and interquartile range (IQR) were calculated for continuous variables. Group differences are examined using t-test and Mann–Whitney U test for parameters with normal and non-normal distributions, respectively. Chi-square or Fisher's exact test was used to test group differences of proportions. A value $p<0.05$ was considered statistically significant throughout.

The kappa (κ) coefficient was used as a measure of agreement between the referring and our expert cardiac pathologist. The strength of the agreement was designed as poor (κ < 0), slight (κ = 0-0.20), fair (κ = 0.21-0.40), moderate (κ = 0.41-0.60), substantial (κ = 0.61-0.80), and near-perfect to perfect (κ = 0.81 to 1.0).

3.5 Results

The cohort (n=158) was predominantly male (n=66%). The mean age was 32 years, age range <1-98 years and 58% were ≤35 years. The majority of deaths occurred at home (n=90, 57%) and most died at rest or during sleep (n=54). Sudden cardiac death occurred during or immediately after exertion in 14% of the total cohort, most of whom were young with a mean age of 24 years.

3.5.1 Causes of sudden cardiac death

A morphologically normal heart, consistent with a SADS death was the predominant finding of our cardiac pathologist accounting for just over 50% of the cases. In over one third (37%) of the cohort a cardiomyopathy was identified. The most common cardiomyopathies included: idiopathic LVH of whom 42% were associated with fibrosis, HCM, ARVC, and obesity cardiomyopathy. Obesity cardiomyopathy affected predominantly those aged over 35 years. However, a third (10/29) were young with the youngest victim aged 9 years with a BMI of 33.1 kg/m^2. Myocardial inflammation was noted in 5 patients. The inflammatory types were: lymphocytic (n=2), toxic (n=1), eosinophilic and lymphocytic (n=1), granulomatous cardiac sarcoid (n=1) and eosinophillic (n=1). Valvular pathology comprised predominantly of mitral valve prolapse (n=3) which was associated with left ventricular fibrosis in 2 cases, followed by bicuspid aortic valve (n=2). Other pathology included congenital cardiac disease, coronary artery pathology, hypertensive heart disease, aortic dissection and cardiac tumours (Table 9).

3.5.2 Specialist cardiac pathology analysis compared to coronial pathology

In 94 of the 158 cases (60%) the provisional diagnosis made by the referring pathologist matched the diagnosis of our cardiac pathologist (Table 9). The κ coefficient as a statistical measure of concordance was moderately significant at 0.48. Consensus was greatest in the diagnosis of DCM, where all 6 cases assigned as DCM by the referring pathologist were confirmed by our cardiac pathologist.

Referring pathologists were more inclined to diagnose pathology rather than designate the heart as morphologically normal, with only 50 out of 80 normal hearts being described as

such. Of the 30 normal hearts diagnosed with pathology by the referring pathologist, 20 were thought to have signs of cardiomyopathy, the majority being ARVC. Of the 21 cases assigned a diagnosis of ARVC by the referring pathologist, 13 were reassigned to a morphologically normal heart after evaluation by our pathologist. The main observation mis-attributed to ARVC was isolated fatty infiltration of the right ventricle (n=10), which is considered a normal finding in the absence of myocardial fibrosis. Myocarditis was also over-reported by the referring pathologist, with 4 out of the 9 cases reassigned to a morphologically normal heart by our cardiac pathologist. In the majority of cases inflammation was focal with no necrosis and/or degeneration of adjacent myocytes. In 2 out of 5 cases of valvular disease over-interpretation of floppy mitral was noted especially in older patients where slight ballooning of the mitral leaflet edges is a normal finding. Both cases were reassigned as normal heart. Finally, coronary artery atheroma was considered a significant cause of death in 3 cases included under the heading of other pathology by the referring pathologist but was determined to be non-significant by our expert cardiac pathologist, highlighting the issue of over-interpretation of the significance of coronary atheroma in the collapsed coronary arteries at autopsy.

Similarly, HCM was over-diagnosed in 10 cases. The referring pathologists had either assigned a diagnosis of HCM in cases of isolated LVH or over-interpreted the extent of myocyte disarray due to sampling of the anteroseptal and posteroseptal walls. Isolated myocyte disarray confined to the anteroseptal and posteroseptal junctions should be considered a normal finding. Five of the 17 cases diagnosed as HCM by the referring pathologist were reassigned to idiopathic LVH by the specialist pathologist (Table 9).

Table 9: Comparison of pathological diagnosis in 158 cases of sudden cardiac death where both the referring pathologists (vertical columns) and the expert cardiac pathologist (horizontal columns) performed a post-mortem cardiac evaluation. The shaded boxes indicate those cases where there was agreement.

	Referring pathologist opinion										
Cardiac pathologist opinion	ARVC	HCM	LVH	DCM	CM NOS	Other CM	Inflammation	Valve disease	Normal heart	Other pathology	**Total**
ARVC	2	2	1	0	0	0	0	0	0	0	**5**
HCM	0	7	1	0	0	0	0	0	1	2	**11**
LVH	1	5	9	0	0	0	0	0	1	0	**16**
DCM	0	0	0	6	0	0	0	0	0	2	**8**
CM NOS	0	0	0	0	4	0	0	0	0	0	**4**
Other CM	3	1	1	0	0	6	1	0	2	1	**15**
Inflammation	0	0	1	0	0	0	4	0	0	0	**5**
Valvular disease	0	0	0	0	0	0	0	3	1	1	**5**
Normal	13	1	2	0	1	3	4	2	50	4	**80**
Other pathology	2	1	0	0	0	1	0	0	2	3	**9**
Total	**21**	**17**	**15**	**6**	**5**	**10**	**9**	**5**	**57**	**13**	**158**

ARVC: Arrhythmogenic right ventricular cardiomyopathy; CM: cardiomyopathy; DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy; LVH: Left ventricular hypertrophy; NOS: Non-specific;

3.6 Discussion

This study reported the results of a unique specialist cardiac pathology service dedicated to the pathological investigation of SCD. To the best of our knowledge this is the first study to compare the results of autopsies as reported by a general pathologist and an expert cardiac pathologist. Our study demonstrates considerable differences in the interpretation of histopathological findings. Coronial general pathologists had the tendency to over diagnose pathology. Of the 74 individuals whose death was attributed to underlying cardiomyopathy by the general pathologist, 20 (24%) cases were reassigned to a

diagnosis of a normal heart by the specialist cardiac pathologist. The majority of those cases (n=13 out of 20) were due to over diagnosis of fatty infiltration and mild right ventricular dilatation for ARVC. The same was true for the presence of myocardial inflammation and valvular disease since almost 50% of the cases (4 out of 9 cases of inflammation; 2 out of 5 cases of valvular disease) were considered to represent normal findings by the cardiac pathologist (Table 9).

3.6.1 Clinical implications

Accurate interpretation of the pathological findings is of utmost importance in order to determine whether the death could be attributed to a potentially inherited cardiac condition. Based on available evidence[81,82] the Department of Health has produced guidelines[8] recommending the referral of all first-degree relatives of victims of SCD due to a potentially inherited cardiac pathology to specialist clinics, in an attempt to identify carriers of quiescent disease and prevent further tragedies. The pathologists report is likely to determine whether surviving relatives will be referred for comprehensive evaluation in a specialist cardiogenetics clinic and guide such a screening process towards structural disorders or primary arrhythmogenic syndromes.

Based on the results of the study, 6 families may have not been referred for further evaluation based on a diagnosis of myocarditis (n=4) and valvular pathology (n=2) from the general pathologist. Both myocardial inflammation and valve disease have been correlated with SCD[14] and in most cases are considered acquired or congenital disorders that are unlikely to affect other family members. On the contrary, however, reassigning the diagnosis to a normal heart by our expert cardiac pathologist, would by definition imply a SADS death with a recommendation for comprehensive evaluation of all first-degree

relatives of the deceased for primary arrhythmogenic syndromes. Moreover, an erroneous diagnosis of cardiomyopathy by the general pathologist in 20 cases may have misled clinicians and led to missed diagnostic opportunities. This is particularly the case in well-established cardiomyopathies such as HCM and DCM, where a normal 12-lead ECG and transthoracic echocardiogram may suffice as first line screening, negating the need for further evaluation for possible arrhythmogenic syndromes.

3.6.2 The importance of a cardiac pathology service

Concerns over autopsies undertaken on behalf of UK coroners have been reported. In a quarter of investigated cases the cardiac autopsy was not of sufficient standard.[79] In recent times pathologists have received limited exposure to autopsy work due to the decline in the hospital autopsy and may be unfamiliar with the spectrum of normal variations such as those associated with gender and age where fatty infiltration of the right ventricle and slight ballooning of the mitral valve can be misinterpreted as pathological. The diagnostic discrepancies identified in the present study and the potential clinical implications underscore the need for an expert cardiac pathology service to support general pathologists in the evaluation of sudden death, particularly in the young. Such a service will ensure a higher probability of accurate interpretation of autopsy findings.

A specialist cardiac pathologist service is also particularly pertinent to primary care physicians who are the first port of call for such families and are required to interpret a post-mortem report, often without any guidance, and initiate referral of family members for cardiological evaluation.

3.6.3 Limitations

The aim of this study was to investigate the importance of expert opinion in the diagnosis of SCD. It is plausible however that some of the referring pathologists intended to send the heart for specialist review from the outset and as such they performed limited histopathological evaluation. Additionally, discrepancies reported between the autopsy conclusions of our specialist centre and that of local pathologists may have been overestimated. The over-diagnosis of ARVC by referring pathologists may in part be explained by the time frame in which this study was conducted where the older task force criteria for ARVC were in effect.[83] Although the 1994 criteria did use the term “fibrofatty” replacement of the myocardium, the presence of fat in the RV myocardium was still considered as the primary diagnostic feature of ARVC. The revised 2010 diagnostic criteria emphasise that histology must confirm the presence of fibrosis, which is now considered the primary diagnostic feature of ARVC, alone or in combination with fatty infiltration.[84] Our centre adhered to the new criteria throughout the study, long before their official acknowledgement.[44]

As with chapter 2, the diagnostic criteria reported in table 5 are derived based on National and International recommendations but also based on the extensive experience of our cardiac pathologist (MNS). As a result certain definitions, such as the differentiation of HCM from idiopathic LVH, may be considered arbitrary rather than evidence based. Moreover, the CRY Centre for Cardiac Pathology service is based upon analysis undertaken by a single pathologist, albeit an acknowledged international expert. Having an additional pathologist to corroborate the expert interpretation would be desirable but single centres cannot justify more than one pathologist doing cardiac work full time. Moreover, there are also few cardiac pathology specialists in the UK. This issue is being addressed

by the UK Cardiac Pathology Network by establishing cardiac pathology pathways in the country to support and train general pathologists.

Chapter 4: Evaluation of families affected by sudden arrhythmic death syndrome – Clinical evaluation of relatives

4.1 Introduction

The finding of a normal post-mortem examination in individuals with no co-morbidities who passed away unexpectedly led to the assumption that such deaths were due to cardiac arrhythmias. As such the term sudden adult death syndrome was proposed, which has been used in death certification in the UK for more than a decade.[3] Behr et al.[85] ware the first to test the hypothesis that a proportion of these deaths may be attributed to primary arrhythmogenic syndromes such as long-QT syndrome (LQTS), Brugada syndrome (BrS) and catecholaminergic polymorphic ventricular tachycardia (CPVT). Given the inherited nature of these conditions, most of which are inherited in an autosomal dominant mode, Behr et al. stipulated that investigating first-degree relatives of the deceased should identify individuals with quiescent cardiac pathology and therefore a probable cause of death. Most importantly, however, identifying asymptomatic individuals with a primary arrhythmogenic syndrome would lead to risk assessment and prevention of further tragedies within the same families.

4.1.1 Studies in sudden arrhythmic death syndrome that formed the basis of the thesis

The study by Behr et al. was the first to utilize the term sudden arrhythmic death syndrome (SADS). The authors invited for limited cardiological evaluation all first-degree relatives of

32 individuals who died and fulfilled the following inclusion criteria: 1. white; 2. aged 4–64 years; 3. no cardiac history; 4. seen alive in the 12 h before death; 5. normal coroner's autopsy and cardiac pathologist's confirmation of normal heart; and 6. negative toxicological screen. Of the potential 147 relatives, 109 (74%) consented to the study. The evaluation included historical review, physical examination, a 12-lead electrocardiogram (ECG) (98%), echocardiogram (97%), and 48-h Holter monitoring (77%). Cardio-pulmonary exercise testing was done on a discretionary basis (13%). Of the 32 families, 6 (19%) were diagnosed with familial cardiac disease based on the identification of a diagnostic phenotype in at least one of the relatives evaluated. Primary arrhythmogenic syndromes and in particular LQTS was the predominant diagnosis: 4 with probable LQTS; 1 with hypertrophic cardiomyopathy (HCM); and 1 with myotonic dystrophy and conductive tissue disease. Overall 6 of the 109 (5.5%) evaluated relatives were diagnosed with a previously unsuspected condition. The authors were also able to extract DNA from the deceased in the 4 families diagnosed with probable LQTS. Genes implicated in LQTS (KCNQ1, KCNH2, KCNE1, KCNE2, and SCN5A) were analysed for disease-causing mutations. Surprisingly however, no mutations were identified.

This research paper was the first to establish a connection between SADS and inherited cardiac disease and in particular primary arrhythmogenic syndromes. It formed the basis for further studies including the main study of the thesis. The paper however, exhibited several limitations that are worth mentioning. The small number of families evaluated and diagnosed with a condition did not allow for any safe conclusions relating to the validity of the methodology utilized. The relatives were subjected to a limited number of investigations, most probably accounting for the very low diagnostic yield. It is highly likely that in the absence of provocative testing with a class-1 antiarrhythmic and exercise testing the authors would have been unable to identify individuals with an underlying Brs

and CPVT. Finally, all LQTS familial diagnoses were based on a single relative identified with a probable diagnosis based on the Schwartz score[86] and the authors failed to identify a LQTS related mutation in the deceased's DNA in those families, casting some doubt as to accuracy of the diagnoses.

A study in Netherlands by Tan et al.[81] investigated 43 consecutive families affected by sudden death. In this particular study a normal post-mortem was not a prerequisite. In fact only in 22 (51%) families had the deceased undergone a post-mortem. The authors evaluated 183 surviving relatives with history, examination, 12-lead ECG, exercise test, transthoracic echocardiography and serum lipid analysis. In cases where BrS was suspected an Ajmaline provocation was performed and when ARVC was suspected the individual was subjected to cardiac magnetic resonance imaging (CMR). Relatives diagnosed with a clinical phenotype of cardiac disease were invited to undergo genetic testing for the relevant implicated mutations.

An inherited cardiac disease was identified in 17 of the 43 (40%) families. Most importantly however, out of the 333 relatives evaluated in the study, 151 were diagnosed with an inherited cardiac disease. As in the study of Behr et al., a diagnosis of primary arrhythmogenic syndrome was the most common finding being present in 12 (71%) families (CPVT: n=5, LQTS: n=4, BrS: n=2, LQTS/BrS: n=1). Four families received a diagnosis of cardiomyopathy (ARVC: n=3, HCM: n=1) and one family was diagnosed with familial hyperlipidaemia. Molecular genetic analysis confirmed the diagnosis in 10 families by revealing mutations in the causative genes. The authors attempted to identify clinical predictors of establishing a diagnosis. The number of investigated relatives was significantly larger in the families in which a diagnosis was established than in those without a diagnosis. Furthermore, the proportion of families with >1 victims was larger

among families with than among those without a diagnosis. Based on those predictors, it is likely that the higher diagnostic yield in the study by Tan et al. compared to the original study of Behr et al. was partly due to the highly malignant family history with an average of 1.8 deaths per family and the high number of relatives evaluated with a mean of 4.3 relatives per family. Additionally however, the more extensive investigations and particularly the inclusion of exercise testing and Ajmaline provocation testing identified conditions such as CPVT and BrS, which are likely to have been missed in the study by Behr et al. The diagnostic yield is even more impressive if one considers that it may represent an underestimate, since only a limited number of families were subjected to Ajmaline provocation testing and as such the authors may have failed to identify some families/individuals harboring the Brugada phenotype.

4.1.2 Studies in sudden arrhythmic death syndrome published during the thesis

In a second study, Behr et al.[82] investigated a further group of unselected families consecutively referred to St George's Hospital in which a SADS death had occurred. The authors hypothesized that a more comprehensive clinical and genetic evaluation of both the SADS victims and surviving relatives would identify a greater proportion of inherited cardiac diseases that could account for the deaths, as well as a greater number of relatives at risk of the same fate. In contrast to the study by Tan et al.[81] where half of the victims of sudden death did not have a post-mortem, Behr et al. placed particular emphasis on the presence of a normal full coroner's post-mortem and negative toxicology screen. To ensure that no subtle cardiac abnormality was missed, the authors endeavored for all hearts or at least tissue samples or slides to be reviewed by an expert cardiac pathologist, and they managed to do so in 75% of the cases. During the familial evaluation the authors obtained as much information as possible for the deceased, including

demographics, mode of death and antecedent symptoms. They also utilised a “molecular autopsy” approach. When available in sufficient quality and quantity, DNA from the deceased was tested for common pathogenic mutations of primary arrhthmogenic syndromes implicated in SADS such as LQTS, BrS and CPVT. Unfortunately, the quality and quantity of this DNA and subsequent mutation analysis was often limited by the nature of retained tissue paraffin blocks. Relatives were subjected to an array of investigations including history, physical examination, 12-lead ECG, transthoracic echocardiogram, exercise treadmill testing and 24-hour Holter monitor. Where a suspicion of cardiomyopathy was raised, the individual was subjected to a CMR. If initial investigations failed to identify any underlying cardiac pathology, relatives were subjected to an Ajmaline provocation test in an attempt to unmask the Brugada phenotype. Further evaluation was guided by initial results. All relatives identified with a clinical phenotype of a potentially inherited cardiac disease were offered genetic testing for the respective condition.

Of the 57 families included in the study, 30 (53%) received a definite or possible/probable diagnosis of inherited cardiac condition. In one of these families clinical evaluation did not contribute to a diagnosis but mutation analysis of the proband’s DNA identified a likely LQTS disease causing mutation. As in the previous studies, primary arrhythmogenic syndromes comprised the majority of diagnoses (21 of the 30 families) with LQTS identified in 16 and BrS in 5 families. In the 9 remaining families a cardiomyopathy was diagnosed (ARVC: n=5, isolated left ventricular non-compaction (LVNC): n=2, HCM: n=1, DCM: n=1). Of the 222 relatives evaluated in the study, 61 (27%) were diagnosed with a previously unsuspected inherited cardiac condition predisposing to sudden death. Subsequent genetic analysis identified disease-causing mutations in 8 of the 30 families. The authors attempted to identify potential predictors of a positive diagnosis by comparing families and individuals with a positive diagnosis versus those where cardiac evaluation

was negative. The only statistically significant predictors were the presence of symptoms in the relatives and, in particular, the presence of syncope prior to evaluation. This is relevant since the authors made the observation that 20% of the SADS victims and 7% of surviving relatives had experienced at least one episode of syncope prior to the death that prompted the familial evaluation. Furthermore, one quarter of families had a history of additional premature (<45 years) sudden deaths, which had not prompted familial evaluation, underscoring the potentially missed opportunities to establish a diagnosis earlier and prevent SADS.

A second study by the Netherlands group,[87] sought to compare the yield of comprehensive clinical and genetic evaluation in surviving relatives of sudden unexpected death victims aged 1–50 years and in victims of aborted cardiac arrest of the same age. Part of the study had been reported before by Tan et al.,[81] as described above. This study used the same definition for sudden unexplained death, which included individuals who either did not have a post-mortem examination or victims with a normal post-mortem examination. The authors hypothesised that survivors of cardiac arrest are likely to be affected by similar conditions to those resulting in sudden unexplained death since the eventual outcome of cardiac arrest is mostly determined by the circumstances at the time of arrest. Of the 140 families with a sudden unexplained death, 33% (n=47) were diagnosed with a certain or probable condition accounting for the sudden death. In 32% (n=45) of the cases a potentially inherited cardiac condition was identified. Two cases of myocarditis were established by revision of the autopsy. Primary arrhythmogenic syndromes were the predominant diagnosis, accounting for 60% (n=27 of 45 diagnoses) of inherited cardiac diseases. Cardiomyopathies accounted for 20% (n=9) of deaths and familial hyperlipidaemia for 13% (n=6). The risk locus at chromosome 7q36, which is associated which familial idiopathic ventricular fibrillation, was identified in 9% (n=4) of the families but

it is unclear as to how many families were investigated for such a diagnosis. Predictors of a positive diagnosis included multiple deaths in the family and the age of the victim. The diagnostic yield was higher in families where the victim was of a younger age, being 71% in the age group 0-10 years compared to only 21% in the age group 41-49 years.

In the 69 victims of aborted cardiac arrest, the diagnostic yield was 61%. Potentially inherited cardiac conditions were present in 45% (n=31) of families, with cardiomyopathies (19%; n=13) and primary arrhythmogenic syndromes (17%; n=12) accounting for equal proportions. The familial idiopathic ventricular fibrillation locus was identified in 6% (n=4) of the cases. 14% (n=6) of arrests were attributed to myocardial infarction and 1.5% (n=1) to mitral valve prolapse. The study did not identify any significant predictors of a positive diagnosis in survivors of cardiac arrest.

The study by van der Werf et al. provides important clues as to the potential value of family screening of victims of sudden unexplained death. Based on the results of the comprehensive evaluation of the survivors of cardiac arrest, screening of first-degree relatives could potentially identify an inherited cardiac condition, accounting for the cause of the death, in up to 45% of families. It also highlights however, our limitations in knowledge and investigative techniques since even comprehensive investigations of the survivors of cardiac arrest failed to identify a cause in almost 40% of the cases. The study however also exhibits important limitations worth mentioning. Given the novel area investigated and the long-duration of this observational study (13 years), investigative tests, the interpretation of such tests and the diagnostic criteria for different conditions are highly likely to have changed with time. As such not all families or survivors of cardiac arrest were evaluated with a single study protocol. This is likely to have caused a considerable underestimation of the true diagnostic yield. In particular, pharmacologic

challenge to diagnose Brugada syndrome and epinephrine challenge were performed only in a selected minority in which no diagnosis was made.

Hendrix et al.[88] also reported on a small cohort of victims and survivors of SCD from a single centre in Netherlands. The authors retrospectively identified potential deaths representing SCDs and reviewed autopsy results and results of the evaluation of family relatives. At the discretion of the authors, families that had not been subjected to all necessary investigations were recalled and re-evaluated. Of the 20 families identified, 5 were attributed to myocardial infarction, 4 to inherited cardiomyopathies, 2 to primary arrhythmogenic syndromes and 1 to aortic stenosis. The cardiogenetic screening of 37 relatives of 12 victims led to a diagnosis of BrS in 3 relatives and the suspicion of ARVC in 2 relatives. The yield of screening of these relatives was 14%. The conclusions that can be derived from this study are fairly limited due to its methodology. Familial evaluation was fairly limited and did not include pharmacological provocation testing, which is likely to have led to underestimation of the contribution of primary arrhythmogenic syndromes such as BrS and LQTS. In 25% of the families no cardiac screening was performed. Moreover, the authors performed the screening themselves in only 4 families, as such it is likely that there was considerable variation in the interpretation of results depending on the physician evaluating the family.

A study by Caldwell et al.[89] reported the experience of a tertiary referral centre of evaluating families affected by sudden unexplained death or aborted cardiac arrest in Manchester. Evaluation of 193 consecutive individuals of 108 families identified an inherited cause of sudden death in 23% (n=45) of individuals from 35% (n=38) of evaluated families. Of the 84 families where a post-mortem had been performed and did not identify any cardiac pathology (SADS deaths), 30% (n=25) of families were diagnosed

with an inheritable cause of sudden death, based on the identification of the clinical phenotype in at least one of the relatives. A total of 31 out of 146 relatives of SADS victims evaluated were diagnosed with an inherited cardiac disease. Primary arrhythmogenic syndromes were the predominant diagnosis (n=19), followed by cardiomyopathies (n=12). Genetic testing identified pathogenic mutations in 12 relatives.

This study highlights the important role of the inherited cardiac diseases clinics in risk modification. Of the 31 relatives identified with an inherited cardiac disease, 21 were initiated on medical therapy: 21 on beta-blockers (10 for LQTS, 4 for CPVT, 3 for HCM and 4 for DCM), 4 on angiotensin converting enzyme inhibitors (ACE-i) for DCM and 1 on Spironolactone. Only 1 individual with a diagnosis of LQTS was implanted with a permanent pacemaker system. Although no individual was implanted with an intracardiac cardioverter defibrillator (ICD), 6 individuals had already an ICD by the time of their referral to the specialist inherited cardiac diseases clinic. On the other hand, the study also underscores potential pitfalls when assessing individuals in the context of a family history of sudden death, where physicians may be more prone to over-interpret results of investigations or offer aggressive device therapy as a result of overestimating the potential risk of SCD. Four individuals had treatment withdrawn after negative genetic testing for an established familial mutation. In one family with a very strong family history of sudden unexpected death (4 out of the 5 siblings of the mother had died at a young age (18-22 years) with a normal post-mortem) the mother and her two sons were commenced on beta-blockade, despite the fact that no cardiac disease had been identified on clinical evaluation. The mother and one of her sons elected to have an ICD implanted. Neither had any appropriate shocks, but the son had inappropriate shocks due to lead fracture. Subsequently, an RYR2 mutation was discovered in an asymptomatic maternal grandfather of the two boys and in tissue from one of the deceased aunts. Genetic testing

showed the mother to be negative for this mutation. Beta-blockers were withdrawn and both ICDs were inactivated/removed. No deaths occurred in relatives diagnosed with a condition or relatives with a familial diagnosis during a short-term follow-up of 2 years. This however is not surprising given the low rates of sudden death and the short follow-up period. The follow-up study did not include any of the families with negative evaluation, which would probably be the group of most interest and would give an indication for potential missed diagnoses.

4.1.3 Isolated genetic screening of family relatives

Unlike the majority of studies evaluating family relatives of SADS victims where genetic analysis complemented clinical evaluation,[90] Wisten et al.[91] utilised genetic screening alone. The authors screened DNA from one or more first-degree relatives of SADS cases for evidence of mutations in the five major LQTS genes, irrespective of familial phenotype. They identified pathogenic LQTS-associated mutations in family members in 3 of the 25 (12%) cases. This yield is not that dissimilar to the yield of LQTS reported by a number of studies after clinical evaluation alone.[81,85,87] The same investigators screened for RyR2 mutations in families without LQTS-associated mutations and found no additional yield.[91] Although this may be a chance finding given the small study sample, it may alternatively represent the higher prevalence of de-novo mutations amongst those affected by CPVT.[92] A similar family genetic screening protocol by Allegue et al.[93] found four LQTS associated mutations (11%) in a series of 35 SADS cases.

4.1.4 The role of molecular autopsy in sudden arrhythmic death syndrome

Molecular autopsy refers to post-mortem genetic analysis of specific candidate genes in an attempt to identify pathogenic mutations that may account for the SADS death. Most importantly however, if such a mutation is identified in the deceased, cascade screening of surviving relatives for the same mutation can be performed in order to identify those at potential risk of the same fate.[90] It is important to note, however, that the presence of a disease-associated mutation does not equate to phenotypic expression of disease and therefore does not necessarily prove a causal relationship between the identified mutations and SADS. Moreover, caution needs to be exercised, as recent studies assessing the prevalence of rare genetic variants in patients and healthy controls have raised concerns relating to the pathogenicity of previously considered disease-causing mutations and highlighted the concept of "genetic noise".[94,95]

Different candidate genes have been investigated in different studies. These include genes for CPVT – RyR2; LQTS – KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2; and BrS – SCN5A.[96-102] Tester et al.[96] sought to determine the prevalence and spectrum of LQTS associated mutations in 49 cases of SADS referred to the Mayo clinic's genomic laboratory. The investigators had previously identified 7 cases of CPVT associated mutations in the same cohort.[103] No victim of SADS or any of their relatives had received a diagnosis of an inherited cardiac disease at the time of the study. Genomic DNA was extracted from frozen necropsy tissue or autopsy blood. Genetic testing for LQTS susceptibility mutations showed 10 putative SADS associated ion-channel mutations/polymorphisms in 10 cases (20%). In 7 cases the authors had the opportunity to perform genetic screening for the identified mutations in relatives of the deceased. The identified mutations were familial in all 7 cases, and a total of 23 genotype-positive family

members were identified. If we account for the previously identified CPVT mutations, the overall diagnostic yield of disease-associated mutations probably accounting for the sudden death, of molecular autopsy increased to 34%. A larger study utilising the same methodology by the same group, tested 173 samples of SADS victims for genes implicated in LQTS and CPVT.[97] On this occasion pathogenic mutations were identified in 45 (26%) of the cases.

A recent population-based study in New Zealand by Skinner et al.[101] identified LQTS pathogenic mutations in 15% (5/33) of victims who underwent molecular autopsy. Another small, retrospective, molecular autopsy series from Denmark revealed a yield of 8.3% (3 of 36) following selective RyR2 sequencing.[100] This compares reasonably with the 12% (20 of 173) yield from Tester et al.[97] All post-mortem studies thus far have used a targeted approach, with up to 64 of the 105 exons of RyR2 being sequenced; the differences in yield may therefore partly be explained by variation in the coding sequence evaluated.

The inclusion of cardiomyopathy associated genes may further increase the yield of molecular autopsy since familial evaluation has recognized that cardiomyopathies underlie some SADS cases with apparently normal hearts.[82] Zhang et al.[99] identified three (12%) possible or probable disease-causing genetic variants in PKP2, the most common ARVC-risk gene, in 25 SADS cases. One (4%) mutation was a ‘radical’ frame-shift mutation strongly associated with disease,[95] strengthening the view that ARVC is responsible for a small, but clinically significant, minority of SADS cases. Hypertrophic cardiomyopathy appears to constitute a smaller proportion of SADS cases on the basis of familial evaluation and appears to have a negligible definitive molecular yield.[93]

Fresh or frozen blood and tissue, particularly spleen tissue provide the best quality DNA for analysis. In the absence of that, alternative sources include formalin-fixed, paraffin-embedded tissue. Unfortunately, formalin fixation causes alterations in DNA sequence. Residual dried blood spot samples from national newborn screening programmes have, therefore, been assessed as a novel source. Gladding et al.[102] extracted DNA successfully from blood spots and used whole-genome amplification prior to sequencing. They were able to perform diagnostic screening of LQTS-risk genes in all 19 cases of their cohort of sudden infant death syndrome (SIDS) and SADS cases, despite some blood spots being up to 39 years old. They showed that six disease-causing mutations identified were all present in at least one blood relative, of whom a proportion exhibited the LQTS phenotype. A retrospective population-based study in Denmark utilising blood-spot derived DNA, reported a yield of LQTS-associated mutations (11%) in 44 cases of SADS.[98]

Alternative technologies have also been utilized to reduce costs: the MassARRAY and SnapShot systems identify pre-specific pathogenic mutations allowing the screening of multiple samples concurrently.[90] They lack the ability, however, to detect novel mutations that are private to a family and are likely to account for a number of cases. Nonetheless, they may be a cost-effective first step in a tiered approach to molecular autopsy in an already well-characterized population.

4.1. 5 Brugada syndrome

A comprehensive background of all conditions implicated in SCD and SADS is beyond the scope of this thesis. Brugada syndrome however, merits special mention as it is a relatively newly described hereditary ion-channel disorder of the cardiac myocytes,[104] and forms a large part of the thesis with chapter 6 focusing on a novel technique for

establishing the diagnosis of BrS and chapter 7 investigates the value of current risk stratification protocols.

Brugada syndrome is characterised by ECG repolarization patterns in the right praecordial leads. [105,106] Three types of ECG patterns are recognised: Type-1 is characterized by a coved ST-segment elevation ≥2 mm (0.2 mV) followed by a negative T-wave; The type-2 ST-segment elevation has a saddleback appearance with a high takeoff ST-segment elevation of ≥2 mm, a trough displaying ≥1 mm ST-segment elevation, and then either a positive or biphasic T-wave; Type-3 has either a saddleback or coved appearance with an ST-segment elevation of <1 mm. To simplify matters, in a recent consensus report of ECG criteria for the diagnosis of BrS the authors suggested the use of only 2 ECG patterns: type-1 identical to classic type-1 of the previous consensus documents (coved pattern) and type-2 that joins types-2 and 3 of the previous consensus.[107] The prevalence of the Brugada ECG pattern is highest in Asia and ranges between 0%–0.36% for type-1 pattern[108-119] and 0%–2.23% for type 2 and type 3 patterns.[120-132] The low prevalence of Brugada-type ECG in the United States may be explained by different ethnic backgrounds among the studies. Of note, there are scarcely any data in subjects of African origin. The prevalence of type 1 ECG in children is reported to be 0.005%, which is much lower than in the adult population.[133] Brugada syndrome is diagnosed only in the presence of a type-1 ST-segment elevation in >1 right praecordial leads (V1-V3) in the presence or absence of a sodium channel blocking agent, and in conjunction with one of the following: documented ventricular fibrillation (VF) or polymorphic ventricular tachycardia (VT), a family history of sudden cardiac death at <45 years old, coved-type ECGs in family members, inducibility of VT with programmed electrical stimulation, syncope, or nocturnal agonal respiration.

The genetic yield in BrS remains poor with a mutation being identified in 15%-30% of clinically affected individuals.[134,135] The first gene linked to BrS was *SCN5A* which encodes the alpha subunit of the cardiac sodium channel (I_{Na}) gene.[106,136] Since 1998 >100 *SCN5A* mutations have been identified in BrS and currently represent the most common genotype, which are predominantly inherited as an autosomal-dominant trait with incomplete penetrance.[137] Other putative causal mutations have also been reported in calcium channel genes (*CACNA1C*, *CACNB2b, CACNA2D1*);[138,139] sodium channel *β*-subunit genes (*SCN1B*, *SCN3B*);[140,141] glycerol-3 phosphate dehydrogenase 1-like enzyme (*GPD1L*) and *MOG1*, which affects trafficking of sodium channels;[134,142-144] and in genes that affect transient outward current (I_{to}) (*KCNE3, KCND3, KCNE5*).[145-147] Functional studies of an *SCN5A* mutation in BrS using heterologous expression systems revealed loss of function of the sodium channel, which impairs the fast upstroke in phase 0 of the action potential and leads to conduction slowing in the heart. Mutations in *CACNA1C* and *CACNB2b* showed loss of function of basal *L*-type calcium *current*; a mutation in *SCN1B*, *SCN3B*, *GPD1L*, or MOG1 led to loss of function of I_{Na}; and a mutation in *KCNE3, KCND3* or *KCNE5* to gain of function of I_{to}.[148] The end-result is an imbalance between the inward and outward currents of the cardiac myocytes and heterogeneous loss of the action potential dome, leading to marked dispersion of repolarisation, re-entrant tachycardias and ventricular fibrillation (VF).[149-151]

The majority of patients with BrS remain asymptomatic throughout their life. Some patients present with syncope and a minority experience sudden death due to VF, which can be the first manifestation. The mean age of death or of aborted VF episodes is 41±15 years. VF episodes occur predominantly in men, who carry a 5.5-fold risk of sudden death compared with women, but arrhythmic events may occur at any age.[106] BrS has also been implicated in sudden death in infants and young children.[137,152] The majority of arrhythmic

events are observed at rest or while asleep.[153,154] Increased vagal tone during night-time is likely to increase the risk of arrhythmic events.[155-158] The potential effect of increased vagal tone in inducing arrhythmias is also supported by the augmentation of ST-segment elevation and unmasking of the diagnostic type-1 Brugada phenotype during the recovery phase of exercise treadmill testing.[159,160] Increased body temperature also appears to induce the Brugada phenotype, malignant arrhythmias and even cardiac arrest which resolves with normalisation of the body temperature.[152,161-165] Supraventricular tachycardias are also fairly common in patients with BrS, with paroxysmal atrial fibrillation observed in 11% to 14% of cases.[166,167]

4.1.6 Provocation testing in Brugada syndrome

The ECG in Brugada gene carriers may be normal or fluctuate between normal and the Brugada pattern.[158,168,169] This has important implications relating to diagnosis in the context of tertiary referral centres, where individuals are likely to be evaluated once or at best on a small number of occasions. It also has important implications relating to prognosis since the presence of the type-1 Brugada phenotype on the baseline ECG is considered a risk factor for malignant arrhythmias and sudden death.[170-176] A study by Veltman et al.[169] evaluated fluctuations of ECG phenotypes in 43 patients with a diagnosis of BrS. The authors performed in total 310 resting ECGs during a median follow-up of 17.7 months. All ECGs were performed at rest and none of the subjects exhibited evidence of hyperpyrexia or extreme vagal tone, which can provoke the Brugada ECG pattern. The patients were compared for different clinical characteristics with respect to the pattern of fluctuations. Out of the 310 ECGs, 102 (33%) revealed the diagnostic type-1 Brugada phenotype, 91 (29%) a saddle-back type-2 phenotype, and 117 (38%) a normal ECG. Only a third of the patients (35%) initially presented with a diagnostic ECG and of those,

only one patient (2%) exhibited constantly the diagnostic ECG pattern during follow-up. Out of 28 patients (65%) with an initially non-diagnostic ECG, eight (19%) developed a diagnostic coved-type ECG during follow-up. Of note, 20 patients (47%) never had a baseline type-1 ECG pattern and the diagnosis was based on developing the diagnostic ECG during Ajmaline challenge. No significant differences were found in gender and clinical characteristics among patients with or without fluctuations between diagnostic and non-diagnostic basal ECGs. The rate of inducible VF was significantly higher in patients with more than 50% coved-type ECGs than in patients with less than 50% diagnostic ECGs, although its value as a risk marker is doubtful as discussed in the next section.

As a result of the dynamic nature of the Brugada ECG pattern, provocation testing with class 1 anti-arrhythmics is usually employed to unmask the Brugada phenotype in suspected cases.[177-180] Although a number of class 1 anti-arrhythmics, such as Ajmaline, Procainamide and Flecainide have been used depending on availability, Ajmaline is the one most commonly utilised in the literature and clinical practice, primarily due to practical considerations. Ajmaline has a considerably shorter half-life compared to Procainamide or Flecainide (several minutes versus 3-4 hours and 20 hours, respectively) and therefore it is eliminated more quickly and requires a short post-test monitoring period. Additionally, Ajmaline appears to have a greater sensitivity. Procainamide displays a more rapid dissociation from the sodium channel and consequently a lower level of use-dependent sodium channel block, which is believed to underlie its lesser potency compared with class 1C agents such as flecainide in unmasking the syndrome. In a study by Wolpert et al.[181] the authors subjected 22 patients with a diagnosis of BrS based on the 2002 diagnostic criteria[105] to both Ajmaline and Flecainide challenge. Ajmaline unmasked the Brugada phenotype in all 22 cases, while Flecainide challenge was negative in 7 (32%) cases. In addition the authors studied the effect of the two drugs on the I_{to} current in canine

ventricular epicardial cells to determine the mechanisms for possible differences in the response to the two sodium channel blockers in patients with BrS. They identified greater inhibition of I_{to} by Flecainide, which may render it less effective.

Ajmaline also has a very favorable safety profile. Out of almost 1,000 Ajmaline tests reported in four studies,[177-180] no deaths were reported. Four individuals experienced persistent VT, which required external defibrillation. In 2 of the 4 cases the Ajmaline test was continued to a target dose of 1mg/kg despite achieving a diagnostic type-1 Brugada phenotype. In one case, the patient exhibited a brief episode of atrial fibrillation that self terminated. Although protocols may differ between different specialist centres, based on the results of these studies, the recommended protocol for Ajmaline administration is for a dose of up to 1mg/kg over 5-10 mins. The Ajmaline challenge should be discontinued if: 1. The diagnostic Brugada pattern is unmasked, 2. The patient develops ventricular ectopy, 3. The patient develops other ventricular or supraventricular arrhythmias and 4. The QRS duration increases >30% compared to the baseline.

Defining the sensitivity and specificity of the Ajmaline provocation test is challenging given the low genetic yield in BrS and the absence of an alternative test that could form the “gold standard” for the diagnosis of BrS. As a result the majority of studies use the presence of type-1 Brugada phenotype with or in the absence of Ajmaline as the main diagnostic test, as per the consensus guidelines.[105,106] Hong et al. [178] studied 147 individuals from 4 large families with SCN5A mutations. The exact SCN5A mutations were not specified. Of the 104 relatives deemed to be at risk of carrying an SCN5A mutation, all underwent a 12-lead ECG and genetic testing. Twenty-four individuals exhibited the type-1 Brugada pattern on the baseline ECG while 71 family members underwent Ajmaline provocation testing. All patients with a positive baseline ECG were mutation carriers. On the contrary, 2 patients

with positive Ajmaline test did not have the mutation, and 7 patients with a negative test had the mutation. Penetrance of the disease phenotype increased from 32.7% to 78.6% with the use of Ajmaline. Although, it is plausible that the 2 individuals with a positive Ajmaline but no SCN5A mutation had a different genetic mutation making them susceptible to SADS due to BrS, based on these results the sensitivity, specificity, and positive and negative predictive values of the Ajmaline test were 80%, 94.4%, 93.3%, and 82.9% respectively. Priori et al.[135] also attempted to define the diagnostic accuracy of pharmacological challenge with sodium channel blockers. Ajmaline or Flecainide provocation test was performed in 8 symptomatic probands and 13 silent gene carriers. The drug challenge unmasked the ECG pattern in only 4 of the 8 probands and 2 of the 13 family members (15%), equating to a positive predictive value of 35%. However, the numbers involved were small and the authors did not specify which drug was used in which patient.

4.2 Aim

The aim of the study was to comprehensively evaluate a large cohort of families referred to the inherited cardiac diseases clinics at King's College Hospital and University Hospital Lewisham from throughout the UK. We attempted to determine: the diagnostic yield at a family level, the diagnostic yield at an individual level and predictors of positive yield. In collaboration with the genetics laboratory at St George's University London we performed testing for gene mutations implicated in ion channel disorders. Finally, in the context of an audit study that primarily aimed to evaluate patient satisfaction (not included in the thesis) we performed a secondary assessment study to detect potential fatalities, other adverse cardiac events and new diagnosis by local specialists.

4.3 Personal contribution

The author performed prospectively the clinical evaluation of the great majority (86%, n=136 of 159) of families, including performing or supervising, analyzing and databasing the majority of investigations (ECG, echocardiography, exercise treadmill test, Ajmaline provocation test and Holter monitoring). Collected data on the deceased including data on previous admissions or GP consultations. Analysed and reported all data.

4.4 Methods

Setting

The SCD of several young individuals and the recognition that a considerable proportion of such deaths are secondary to hereditary conditions, prompted the United Kingdom (UK) government to commission the 8th chapter of the National Service Framework for coronary heart disease, aimed at facilitating early identification of individuals at risk of SCD. King's College Hospital St George's Hospital and University Hospital Lewisham, amongst others, implemented dedicated inherited cardiac diseases clinics, serving relatives of individuals who experienced SCD, from throughout the UK (Figure 8). The primary supervisor of the thesis, Prof Sanjay Sharma, oversees all clinics.

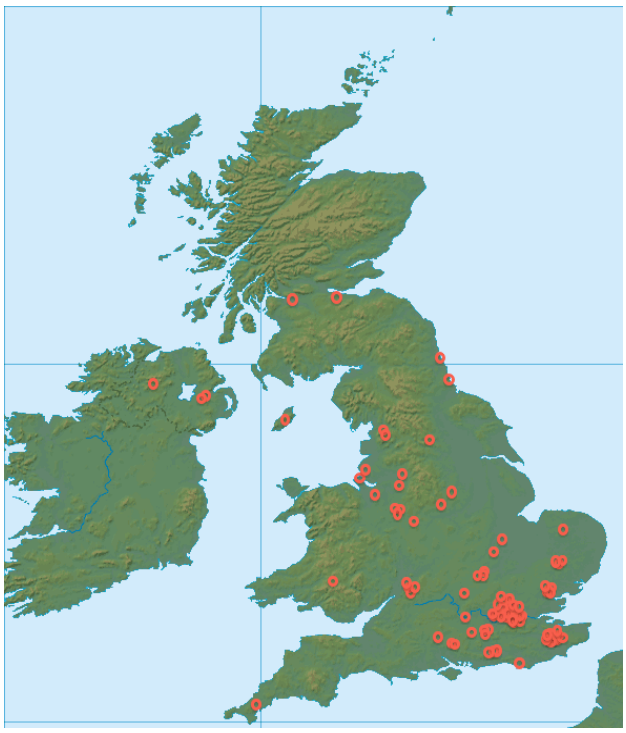


Figure 8: Map of the UK. The red circles indicate geographical regions from which families were referred to our clinic for evaluation after a sudden death. Families would travel from as far as Scotland, Northern Ireland and Cornwall.

4.4.1 Inclusion criteria

Between October 2006 and October 2010, 159 families, comprising 501 individuals, were referred to our clinics for cardiac evaluation after a SCD. Families were considered for inclusion in the study if the deceased fulfilled the following criteria: 1. Unexpected death of an apparently healthy individual with no past cardiac history of note; 2. Death from natural causes; 3. Last seen alive and well within 12 hours; 4. Complete post-mortem report; 5. The absence of an extra-cardiac cause of death; 6. Negative toxicology screen; and 7. The absence of an established diagnosis based on evaluation of relatives by another specialist. Twenty-three families were excluded from further analysis based on: The presence of documented past medical history prior to death (1 with Wolf-Parkinson-White electrocardiographic pattern, 1 with atrial fibrillation); One individual with a history

consistent with Commotio cordis; The absence of a complete post-mortem report (n=6); Positive toxicology (n=8), including the detection of cannabis, cocaine, ecstasy and alcohol; and an established diagnosis of an inherited cardiac condition in family relatives prior to evaluation in our clinic (n=6) (Figure 9).

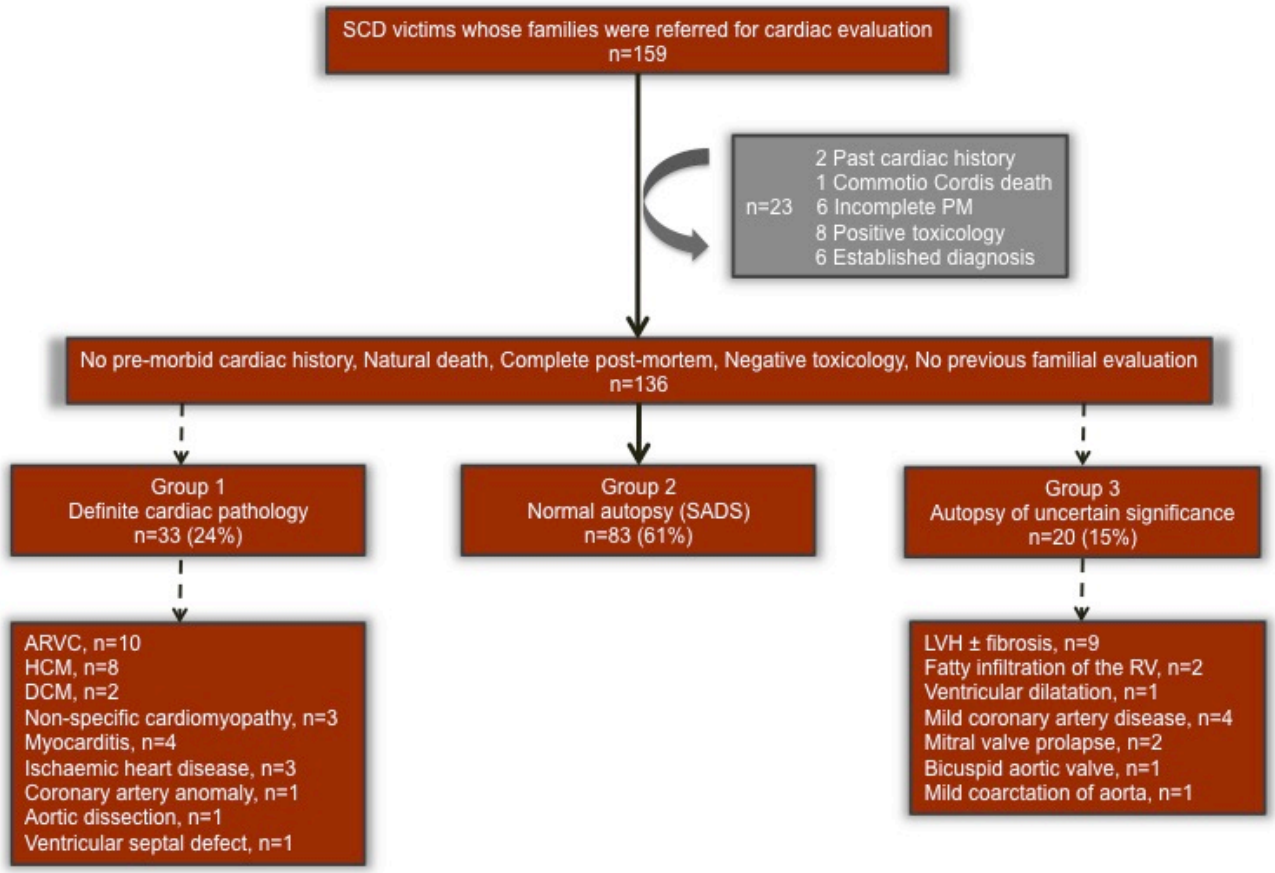


Figure 9: Flow diagram describing the number of families referred to our inherited cardiac disease clinics and their classification according to the post-mortem result.

ARVC: Arrhythmogenic right ventricular cardiomyopathy; DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy; LVH: Left ventricular hypertrophy; SADS: Sudden arrhythmic death syndrome.

The post-mortem reports of the 136 SCD cases were subsequently scrutinized by the author and Prof Sanjay Sharma and divided into three groups: Group 1: Autopsy findings

highly suggestive of structural cardiac pathology accounting for the SCD as defined in table 5; Group 2: No identifiable structural cardiac pathology, consistent with a SADS death; and Group 3: Autopsies of uncertain causal effect (Table 15 in chapter 5). In cases of disagreement a third, senior collaborator was consulted (Prof Sheppard, cardiac pathologist or Dr Behr, consultant electrophysiologist with expertise in SADS). The study cohort consisted of the 83 families in group 2, comprising of 271 blood relatives (Figure 9).

4.4.2 Familial evaluation

The overwhelming majority of families included in the thesis were evaluated at University Hospital Lewisham. In an attempt to minimise the psychological and physical burden of screening on the bereaved families, we adopted a novel one-stop model aiming to evaluate individuals and families during a single session. Our one-stop model allowed for the maximum number of relatives to be evaluated under one roof, without the need for repeated visits. This is of particular importance in the context of inherited cardiac conditions where uncertainties still exist as to the diagnostic accuracy of investigations and clinical outcomes, and results in one family member may affect the interpretation of the results or treatment in another relative.

Our multidisciplinary team consisted of cardiologists with expertise in conditions predisposing to SADS, an arrhythmia nurse and dedicated physiologists. We also collaborated with other leading institutions including the Royal Brompton Hospital and St Thomas' Hospital for cardiac magnetic resonance imaging (CMR), the CRY Centre for Cardiac Pathology at the National Heart and Lung Institute at the Royal Brompton Hospital for detailed histological evaluation of post-mortem cardiac tissue and St George's Hospital for genetic testing of blood samples from family relatives who were clinically diagnosed

with an inherited cardiac condition. The authors also closely collaborated with the charitable organization CRY, which has an extensive network around the UK in order to provide bereavement support to the family and psychological support to relatives diagnosed with a condition predisposing to SADS.

With the consent of the family, the author collected information on the deceased (proband) by review of coroner's reports, autopsy reports, primary care physician's and hospital records, when available, and during interview with the relatives. Information collected included demographics; prior cardiac symptoms, including episodes of chest pain, shortness of breath (including asthma attacks), palpitations, dizziness/pre-syncope and syncope (including epileptic fits); past medical history including hospital admissions, information relating to previous cardiac investigations; family history of sudden death, epilepsy, asthma, unexplained road traffic accidents or drowning and episodes of syncope; and importantly the results of the post-mortem evaluation. When possible, in cases where the post-mortem had not been performed by a cardiac pathologist, with the family's consent and the assistance of CRY, we facilitated the transfer and examination of the heart at Royal Brompton hospital. When available, we collected blood samples and/or spleen tissue for DNA analysis from the deceased as part of an ongoing research project on the contribution of "molecular autopsy", which is not addressed in the current thesis.

Individual family members underwent comprehensive cardiac evaluation aimed at identifying inherited cardiac pathology that may have accounted for the SADS death according to the algorithm depicted in Figure 10. Although we aimed to investigate first-degree relatives prior to evaluating more distant relatives of the deceased, that was not always possible. As such the authors offered screening to any relative referred to our clinic. Evaluation in children <16 years old was limited to ECG and transthoracic

echocardiography. In cases where a familial diagnosis was established and/or the children exhibited high-risk features such as syncope, a paediatric cardiologist performed further comprehensive evaluation. In the great majority of families, results of the 12-lead ECG, transthoracic echocardiography, exercise treadmill testing, CMR and Ajmaline provocation testing were available on the day of the consultation. Holter monitors were placed at the end of the investigations and all were returned back for analysis. During the final consultation, the families were presented with the results and treatment options. After the consultation we offered support to the family including, medical care and psychological support for the ones diagnosed with a condition predisposing to SADS, bereavement support for the family and a portal of contact with our medical and nursing staff for further queries to avoid the need for additional visits.

12-lead electrocardiogram

Digital 10 sec ECGs were acquired during quiet respiration in a supine position using a MAC 5000 (GE Medical, Milwaukee, Wisconsin; 500 Hz, 4.88 mV resolution). The electrodes were placed carefully to ensure consistency, and ECGs were recorded at a paper speed of 25 mm/s. Electrocardiograms were performed with leads V1 and V2 placed in the conventional 4th intercostal space. From October 2007 onwards all ECGs were also recorded with leads V1 and V2 placed in the higher 2^{nd} and/or 3^{rd} intercostal space. Electrocardiograms were subsequently exported to a customised software program where tracings could be enlarged to high magnification and amplitude to measure basic intervals accurately with on-screen callipers. Heart rate and QRS axis were calculated. P-, Q-, R-, S-, and T-wave voltages; ST-segments; QRS duration; PR interval; and QT-interval were measured in each lead. The QT-interval was corrected for the heart rate (QTc) using the Bazett's formula.[182] Left and right axis deviations were defined as a frontal cardiac axis

≤ -30° or ≥ 120° respectively. Left ventricular hypertrophy (LVH) was identified using the Sokolow–Lyon criterion.[183] T-wave inversions in two or more leads were considered significant, excluding leads aVR, V1 and III. T-wave inversions in leads V1-V3, outside the context of symptoms or a familial diagnosis of cardiomyopathy and in particular ARVC, were considered to represent the juvenile ECG pattern in individuals <16 years old.[184] Adolescent individuals with anterior T-wave inversions were monitored serially until adulthood to ensure resolution of the T-wave inversions. Deep T-wave inversion was defined as a negative T-wave of -0.2 mV or more in any lead. Partial right bundle branch block (pRBBB) was defined as QRS duration > 0.1 but < 0.12 seconds, with rSR' morphology in lead V1 and qRS in V6.[185] Additional electrocardiographic markers compatible with ARVC were also sought, including terminal activation duration of the QRS complex ≥ 55ms in leads V1, V2 or V3, and the epsilon (ε) wave.[83,84]

Electrocardiographic recordings from study V were reviewed retrospectively for the presence of early repolarization (ER), based on the results of recent studies which indicated that early repolarization in the inferior and lateral leads may represent a potentially heritable marker of malignant arrhythmias, particularly in the context of SADS.[186-188] Inferior (II, III, AVF) and lateral (I, AVL, V4-V6) leads were assessed for the presence of J-point elevation defined as ≥0.1 mV in ≥2 leads in the same territory. The ER pattern was further classified according to the morphology of the terminal QRS in the majority of the leads as notched, slurred or indeterminate. In the presence of ER, the ST-segments were assessed for the presence of ST-segment elevation and a distinct ST-segment morphology (ascending or horizontal/descending).[186]

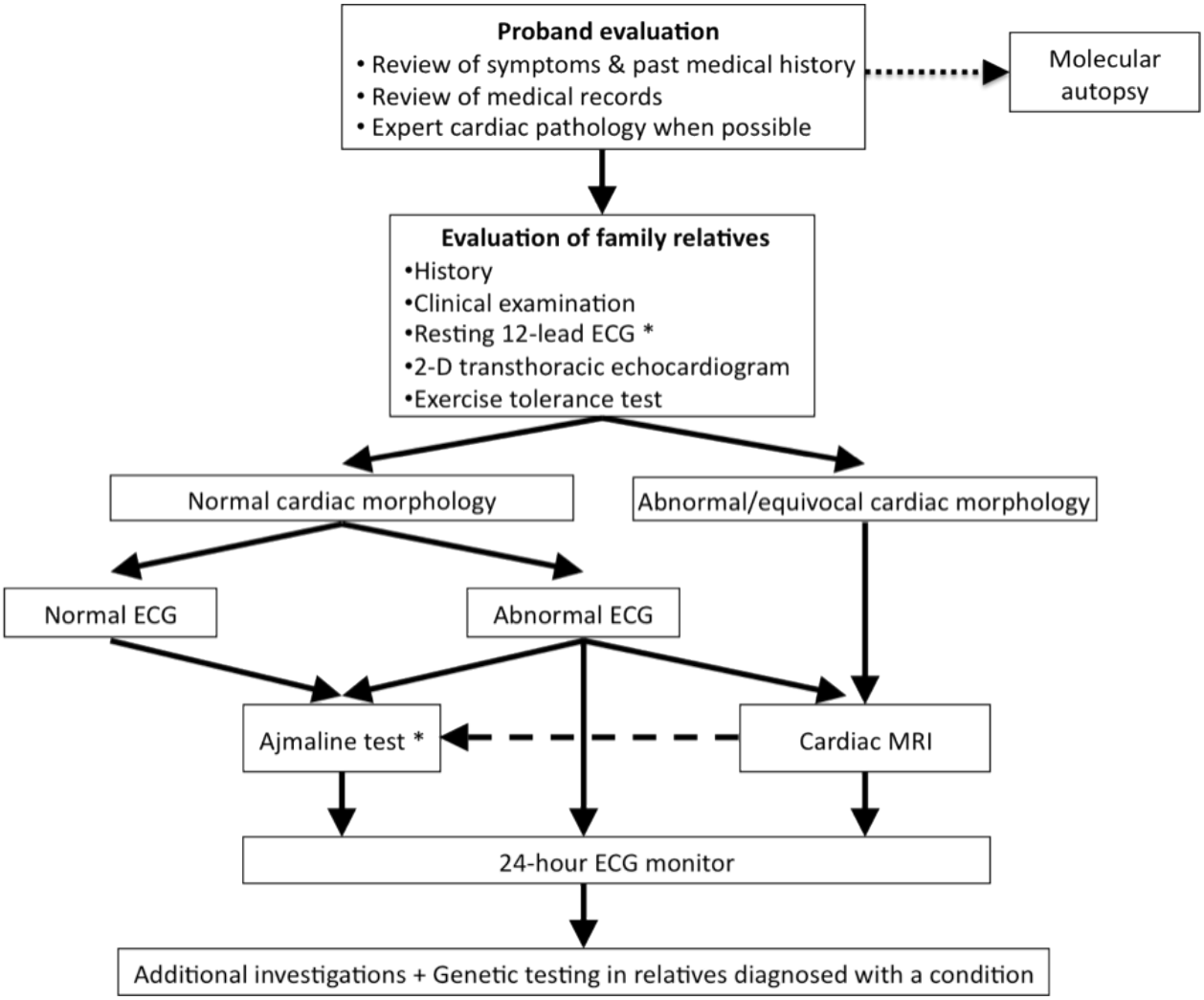


Figure 10: Diagnostic algorithm for families referred with a diagnosis of sudden arrhythmic death syndrome death

* Electrocardiograms were performed with leads V1 and V2 placed in the conventional 4th intercostal space as well as the higher intercostal spaces (2nd and/or 3rd intercostal space)

Echocardiography

Two-dimensional echocardiography was performed by a cardiologist or a senior cardiac physiologist, with the subject at rest, in the left lateral decubitus position using the following commercially available ultrasound systems: GE Vivid I, GE Vivid 7 (GE Healthcare, Milwaukee, WI, USA) and Philips iE33 (Philips Medical, Bothel, Washington, USA). A complete echocardiographic study of the left and right heart was performed

according to guidelines from the European Society of Cardiology and the American Society of Echocardiography.[189,190]

Two-dimensional continuous- and pulsed-Doppler, as well as colour tissue-Doppler imaging were performed using standard parasternal and apical views. Left ventricular wall thickness was measured from two-dimensional short-axis views at end-diastole and the greatest measurement within the left ventricular wall was defined as the maximal wall thickness. The systolic pulmonary artery pressure was estimated using the simplified Bernoulli equation ($4V^2_{max}$ + right atrial pressure) where V_{max} is the maximal velocity of the tricuspid regurgitant jet measured using continuous-wave Doppler.[191] In the absence of a raised jugular venous pressure during cardiovascular examination the right atrial pressure was assumed to be 5 mmHg.

Exercise tolerance testing

All subjects ≥16 years of age who were physically able to exercise were subjected to an upright treadmill test to volitional exhaustion. The investigators utilized the standard Bruce protocol.[192] Signals from a 12-lead ECG were displayed continuously, looking specifically for the development of arrhythmias,[193] ischaemic changes, paradoxical prolongation of the QT interval[194-196] or the Brugada phenotype.[160] Blood pressure was measured by auscultation over the brachial artery at 1 min intervals during the test and for the first 3 min after the test using a manual sphygmomanometer. A systolic blood response of >25 mmHg from baseline to peak exercise is considered normal.[197-201]

24-hour Holter monitoring

Ambulatory ECG monitoring (Lifecard CF Holters, Spacelabs Healthcare, USA) was analysed for any evidence of supra-ventricular and/or ventricular arrhythmias.[202] Individuals were encouraged to continue their usual daily activities, including exercise during the recordings.

Ajmaline provocation test

Ajmaline provocation testing to identify the type-1 Brugada phenotype was performed in the event of normal ECG recordings and echocardiograms or in the presence of type-2 or type-3 Brugada ECG patterns. Selected individuals with a LQTS type-3 ECG phenotype were also subjected to an Ajmaline provocation test due to the documented overlap between the two syndromes.[203-207] Ajmaline provocation test was performed with verbal consent in individuals ≥16 years of age. In all cases an advanced life support certified cardiology trainee and an arrhythmia nurse or cardiac physiologist were present. Standby resuscitation facilities, including isoprenaline,[208-210] were available. Ajmaline was infused either as boluses of 10mg/minute or as a continuous infusion over 5 mins to a target dose of 1mg/kg.[177-180] The test was discontinued prematurely if the patient exhibited significant side effects, such as symptoms secondary to hypotension, developed multiple premature ventricular beats or ventricular arrhythmias or the ECG developed the diagnostic type-1 Brugada pattern in the standard or higher right precordial leads. In contrast to some studies in the literature,[179] QRS prolongation was not utilized as a criterion for discontinuing Ajmaline infusion.

Digital 10 sec ECGs were performed during quiet respiration in a supine position using a MAC 5000 (GE Medical, Milwaukee, Wisconsin; 500 Hz, 4.88 mV resolution) and were acquired at short intervals (every 15 s) during Ajmaline administration and for up to 10 min

after drug administration in the case of a negative test or until all ECG changes had resolved completely in the case of a positive test. All ECGs were subsequently exported to a customised software program where tracings could be enlarged to high magnification and amplitude to measure basic intervals accurately with on-screen callipers. Electrocardiograms were recorded continuously with leads V1 and V2 placed in the conventional 4th intercostal space. All individuals evaluated in our clinic from October 2007 onwards, underwent Ajmaline provocation testing with leads V1 and V2 placed in the higher 2nd intercostal space (217 out of the 261 (83%) Ajmaline provocation tests reported in this thesis). Towards the later part of the thesis, the ECG configuration consisted of the four limb leads, V1 and V2 leads recorded from the 4th, 3rd and 2nd intercostal spaces (six electrodes), and the remaining three electrodes recorded V4-V6, omitting the standard V3 position (75 out of the 261 (29%) Ajmaline provocation tests reported in this thesis), as our experience and recent observations suggested that this lead is rarely positive or central to the diagnosis of BrS.[211] Baseline and maximum drug effect ECGs were analysed. The maximum drug effect was determined based on the broadest QRS width. The maximal J point elevation was measured at baseline and during the maximal drug effect in standard and high right precordial leads.

Cardiac magnetic resonance imaging

All cardiac magnetic resonance imaging (CMR) was performed and analysed at Royal Brompton hospital. All relatives with ECG or echocardiographic features suggestive of cardiomyopathy were subjected to CMR. Cardiac magnetic resonance imaging was performed with a Siemens Sonata 1.5 T (Erlangen, Germany) using steady-state, free precession breath-hold cines (TE/TR 1.6/3.2ms, flip angle 608) in long-axis planes and sequential 7 mm short-axis slices (3 mm gap) from the atrioventricular ring to the apex.

Late gadolinium enhancement images were acquired 10 min after intravenous gadolinium-DTPA (Schering, 0.1mmol/kg) in identical short-axis planes using an inversion-recovery gradient echo sequence. Inversion times were adjusted to null normal myocardium (typically 320–440ms; pixel size 1.7 x 1.4 mm). Late gadolinium enhancement images were phase swapped to exclude artefact. Ventricular volumes and function were measured for both ventricles using standard techniques and analysed using semi-automated software (CMR tools, Cardiovascular Imaging Solutions, London, UK). All volumes and masses were indexed for age, gender, and body surface area (BSA).[212,213]

Further evaluation

Further evaluation was dictated by initial results and included computer tomographic coronary angiography (CTCA), conventional coronary angiography, tilt-table testing and neurological evaluation, and electrophysiological studies.

Genetic testing

Mutation analysis was offered to all relatives with phenotypic abnormalities suggestive of inherited arrhythmogenic syndromes or cardiomyopathies, after appropriate counselling, as recommended by UK guidance.[214] Following appropriate counselling and consent, targeted mutation analysis was performed in one phenotypically affected member of each family, dependent upon the suspected clinical condition. Gene testing was performed for known mutations in the following genes: *KCNQ1, KCNH2, KCNE1, KCNE2* and *SCN5A* in LQTS; *SCN5A* in BrS; selected exons (7–9, 13–16, 43–50, 82–84, and 87–105) of RYR2 in CPVT*; MYH7, MYBPC3, TNNT2, TNNI3 and TPM1* in HCM*; PKP2, DSP, DSC2* and *DSG2* in ARVC; lamin A/C gene (*LMNA*) in dilated cardiomyopathy (DCM) with evidence of conductive tissue disease.

Genomic DNA samples were extracted from peripheral lymphocytes in blood using standard techniques (Nucleon® Genomic DNA Extraction Kit, Tepnel PLC) and normalized to a concentration of 50 ng/ml. Genotyping was carried out using the ABI 3130 System (Applied Biosystems) to investigate potentially novel mutations in exonic regions and intron/exon boundaries. Exons and flanking intronic regions were amplified from genomic deoxyribonucleic acid (DNA), using standard polymerase chain reaction (PCR) techniques with primer sequences by Sigma-Genosys. Amplification was carried out with the use of a Touchdown Thermal Cycler (Hybaid); any product that did not amplify successfully was subjected to a different PCR method using HotStar Taq polymerase (Qiagen) following the manufacturer's protocol. In some cases it was necessary to add 4 µl of a PCR additive, Q solution (Qiagen), in a total 25 µl reaction. Products were bi-directionally sequenced to identify coding variants using the Applied Biosystems kit, v3.1 for a 10µl reaction in an automated capillary DNA Sequencer (ABI3130, Biomics Centre, St George's University of London).

Identified mutations were reviewed by our geneticist relating their association or likely association with disease. If a mutation considered to be pathogenic or likely pathogenic (known or highly probable) was identified in a relative, the family was offered cascade screening. Variants were labeled as pathogenic if they were: previously reported to be associated with disease-susceptibilty;[215-222] in-frame or frameshift-causing insertions or deletions; affecting splice sites; missense mutations likely to be pathogenic, as identified by two in-silico models [affect protein function by a tolerance index score of <0.05 in sorting intolerant from tolerant (SIFT) and classified "probably damaging" by polymorphism phenotyping (PolyPhen)].[223,224]

4.4.3 Diagnostic criteria

Brugada syndrome

Standard diagnostic criteria for the diagnosis of Brugada syndrome were used as proposed in the second consensus document.[106] A diagnosis of definite BrS was only established in the presence of the type-1 Brugada pattern (coved ST-segment elevation ≥2mm followed by a negative T-wave) in >1 right praecordial leads (V1 to V3) in the presence or absence of a sodium channel-blocking agent. The authors utilized the higher intercostal leads, as described in the 12-lead ECG and Ajmaline provocation testing sections, which were also considered diagnostic.[225-227] Given the nature of the study all individuals included had a family history of SCD. The majority of the deceased were <45 years old and in a considerable proportion of families more than one of the relatives investigated exhibited the type-1 Brugada pattern. As part of the familial evaluation, the authors enquired about prior history of syncope, epileptic seizures or nocturnal agonal respiration and attempted to document arrhythmias including polymorphic VT. No individual was subjected to electrophysiological studies for inducible ventricular tachycardia for diagnostic purposes given the controversies relating to the value of the test both as a diagnostic as well as prognostic tool.[170-176]

Long-QT syndrome

Standard LQTS diagnostic criteria (Schwartz score) were utilized for LQTS and the individuals were classified as low, intermediate or high probability of carrying a LQTS mutation.[228]

Short-QT syndrome

Short QT syndrome was considered in individuals with a QTc <320 msec with tall peaked T waves.[229,230]

Catecholaminergic polymorphic ventricular tachycardia

Cathecholaminergic polymorphic ventricular tachycardia was considered in the context of symptoms (palpitations, pre-syncope or syncope) induced by adrenergic surges such as vigorous physical activity or emotional stress. Diagnosis of CPVT required the documentation of polymorphic ventricular tachycardia, but not necessarily typical bidirectional VT with a beat-to-beat 180 degrees rotation of the QRS complex, during exertion and in the absence of ECG features suggestive of an alternative diagnosis.[92,231,232] Epinephrine testing was not utilized during the course of this study and exercise tolerance testing was the predominant diagnostic tool.

Progressive conductive tissue disease

Progressive conductive tissue disease was considered in the presence of premature conduction tissue disease, as evident by the presence of a high degree atrioventricular block on the 12-lead ECG. Other ECG indicators examined included a long p-wave, prolonged PR and QRS intervals and right or left bundle branch block.[233-235]

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy was defined as LVH on transthoracic echocardiography with a maximal end-diastolic left ventricular wall thickness (max-LVWT) ≥15 mm in the absence of a systemic cause such as hypertension or aortic valve stenosis. A max-LVWT <15 mm was also considered diagnostic of HCM in the context of electrocardiographic repolarization anomalies suggestive of the disease and identification of HCM in another relative. Athletic activity, gender and ethnicity was taken into account given the experience of our group which suggests that a considerable proportion of athletes and particularly athletes of African/Afro-Caribbean descent may develop a max-LVWT between 13-16 mm in response to regular exercise.[57,72,236] Other echocardiographic indices assessed included ventricular cavity size, atrial cavity size, the presence of systolic anterior motion of the anterior mitral valve leaflet, the presence of a left ventricular outflow tract gradient at rest or during provocation with Valsava manoeuvre or exercise and systolic and diastolic function indices.[237,238]

Arrhythmogenic right ventricular cardiomyopathy

During the initial stages of the research project the 1994 diagnostic criteria were used for the diagnosis of ARVC.[83] The authors applied the updated 2010 criteria after their publication and retrospectively reviewed all cases raising suspicion of ARVC but which did not fulfill the 1994 criteria.[84]

Dilated cardiomyopathy

A familial form of DCM was suspected in the presence of left ventricular dilatation and left ventricular systolic dysfunction in the absence of abnormal loading conditions such as hypertension or valvular disease and in the absence of significant coronary artery disease.[239] The individual's demographics and athletic activity were taken into account based on evidence and our group's experience suggesting that regular exercise can result in considerable ventricular dilatation as a result of cardiovascular adaptation to exercise, which is more pronounced in males compared to females, in terms of absolute dimensions.[240,241]

Left ventricular non-compaction

Left ventricular non-compaction (LVNC) was considered when prominent left ventricular trabeculations and inter-trabecular recesses were observed on trasthoracic echocardiography.[239] Left ventricular non-compaction was diagnosed on 2-D trasthoracic echocardiograms utilizing both the Chin et al.[242] and Jenni et al criteria.[243] The individual's demographics, athletic activity, any co-morbidities such as sickle-cell anaemia and pregnancy status were taken into account based on published experience from our group suggesting that regular exercise, sickle-cell anaemia and pregnancy can result in pronounced trabeculations of the left ventricular myocardium fulfilling diagnostic criteria for LVNC.[244,245]

4.4.4 Short-term follow-up

We set out to investigate the short-term outcomes of families evaluated in the inherited cardiac diseases clinics and in particular, compare outcomes between families (and individuals) with a diagnosis of an inherited cardiac pathology to families (and individuals) in whom no pathology was identified. The author investigated any morbidity or death resulting after the family's initial assessment in our clinic. We collected follow-up data on individuals under regular follow-up in our clinic. For individuals that were either discharged from our clinic after a negative familial evaluation or were followed-up by a local specialist we sent out a questionnaire as part of a clinical audit evaluating the service provided in our novel inherited cardiac diseases clinics. An introductory letter and a questionnaire were mailed to all individual family members, aged ≥16 years, reviewed in our clinics. For individuals <16 years the letter was addressed to the parent or guardian. Individuals were asked: 1. Any further unexpected deaths in your family since the consultation? 2. Anyone in your family with unexplained fainting episodes since the consultation? 3. Have you or any of your relatives assessed in our clinic been diagnosed with a heart condition since the consultation?

Ethical approval

Data collection was performed as part of the clinical evaluation of the families as proposed by the Government in the 8th chapter of the National Service Framework for Heart Disease, which includes guidelines for early identification of individuals at risk of sudden cardiac death and better support for families of victims. The follow-up project was performed as part of a clinical audit evaluating the services provided in our inherited cardiac diseases clinics. The opinion of the Outer South East London Research Ethics

Committee was sought who recommended that the project represented a service audit and no ethical approval was required.

Statistical analysis

Data interpretation and analyses were performed using SPSS software, version 14 (SPSS Inc., Chicago, IL, USA). Means and standard deviations (SD) or median and interquartile range (IQR) were calculated for continuous variables. Group differences are examined using t-test and Mann–Whitney U test for parameters with normal and non-normal distributions, respectively. Chi-square or Fisher's exact test was used to test group differences of proportions. Binary logistic analysis was used to investigate the presence of an independent association between age, gender, presence of symptoms, number of family relatives evaluated and family history of sudden death and the presence of an underlying cardiac pathology in the families. A value $p<0.05$ was considered statistically significant throughout.

4.5 Results

Out of 159 families referred to the inherited cardiac diseases clinics as a result of a sudden death, 83 families, comprised of 271 blood relatives, fulfilled the SADS criteria (Figure 9).

4.5.1 Characteristics of probands

The majority of SADS probands were male with a male to female ratio of 2.3 : 1 (Table 10). Most deaths (70%) occurred either during sleep or at rest, with only 15% of deaths related to exercise. One of the victim's death was provoked by the alarm clock going off.[246]

A pre-morbid 12-lead ECG and subsequent familial evaluation revealed a diagnosis of LQT type 2 in the proband, her mother and maternal cousin.

Table 10: Probands' characteristics	
Total number of probands	83
Mean age [range] (years)	29 [1-56]
Gender (male)	58 (70%)
Ethnicity: Caucasian Asian Afro-Caribbean	 74 (89%) 6 (7%) 3 (4%)
Mode of death: Asleep At rest Daily activities Exercise	 33 (40%) 25 (30%) 13 (16%) 12 (14%)
Antecedent cardiovascular symptoms: Syncope	30 (37%) 14 (17%)
Family history of sudden death First-degree relative	19 (24%) 10 (12%)
Premorbid ECG: Abnormal	16 (20%) 10 (12%)
Cardiac pathologist post-mortem	38 (47%)

Of importance, 37% of the victims had reported cardiovascular symptoms prior to their death. Syncope (n=14) and palpitations (n=13) were the most common symptoms, followed by episodes of dizziness/pre-syncope (n=6), shortness of breath (n=4) and chest pain (n=1). Of the 30 victims who reported cardiovascular symptoms, only 8 (27%) were investigated by a specialist. In 4 individuals the presenting symptom was witnessed generalised seizures. All 4 were investigated by a neurologist and 2 received a diagnosis

of epilepsy (Table 11); in both cases familial evaluation identified an ion-channel disease, namely BrS and LQTS.

Table 11: Characteristics of the 8 SADS victims who exhibited cardiovascular symptoms that prompted specialist evaluation prior to their death. The table also depicts the results of the familial evaluation prompted by the SADS death.

Gender Age of death	Symptoms	Investigations	Diagnosis Specialist	Treatment	Diagnosis post familial evaluation
Male 31 years	VF arrest	ECG: ST-elevation in lateral leads, ECHO, coronary angiogram (Normal)	Coronary artery spasm Cardiologist	Thrombolysed, Medical therapy, Not for ICD as VF in the context of ischaemia	Negative familial screening Coronary artery spasm
Male 25 years	palpitations	ECG: partial RBBB, minor anterior ST-elevation, Normal ECHO & Holter monitor	?Arrhythmia Cardiologist	Beta-blocker	BrS
Female 26 years	1 episode of generalised seizure	CT head & EEG (Normal)	No diagnosis Neurologist	No treatment	Brs
Female 56 years	1 episode of syncope	CT head (Normal)	Possible TIA Neurologist	No treatment	BrS
Male 23 years	Recurrent episodes generalised seizures	CT head & EEG (Normal), ECG: Type-2 Brugada phenotype in V1 & V2	Epilepsy Neurologist	Sodium Valproate	BrS
Male 32 years	Recurrent episodes of epileptic fits	CT head, EEG (Normal), ECG: prolonged QT (Figure 11A)	Epilepsy Neurologist	Levetiracetam	LQT
Female 17 years	Nocturnal seizures	CT & MRI head, EEG (Normal)	No definitive diagnosis Neurologist	No treatment	BrS
Female 16 years	Palpitations, episodes of pre-syncope	Serial ECGs: T-wave inversions in V1-V3, LBBB VE (Figure 11C) ECHO: Normal, Holter: LBBB VEs	No diagnosis Cardiologist	No treatment	ARVC

Almost a quarter of the victims had a prior family history of SCD at a relatively young age (<50 years), of which a considerable proportion was in first-degree relatives. We were unable to obtain any post-mortem reports of those relatives. Of the 7 death certificates obtained, 4 were attributed to myocardial infarction or coronary artery atherosclerosis, 2 to cardiac arrhythmia and 1 to sudden adult death syndrome. All were associated with a verdict of death from natural causes. None of the families was advised to seek cardiac evaluation for inherited conditions predisposing to SCD.

The author was able to obtain a 12-lead ECG in 16 victims of SADS. The majority of ECGs (14 out of 16) were performed as a result of cardiovascular symptoms. In 2 cases the ECGs were performed as part of routine investigation for unrelated conditions. Six of the ECGs exhibited diagnostic phenotypes, highly suggestive of a condition predisposing to sudden death. One ECG depicted ST-segment elevation in the lateral leads consistent with myocardial ischaemia in an individual with a diagnosis of coronary artery spasm (Table 11). The other 5 ECGs were suggestive of an inherited cardiac condition (LQTS, n=3; BrS, n=1; ARVC, n=1) (Figure 11). In 3 out of these 5 cases, subsequent familial evaluation identified the respective diagnostic phenotypes in ≥1 of the relatives of the deceased.

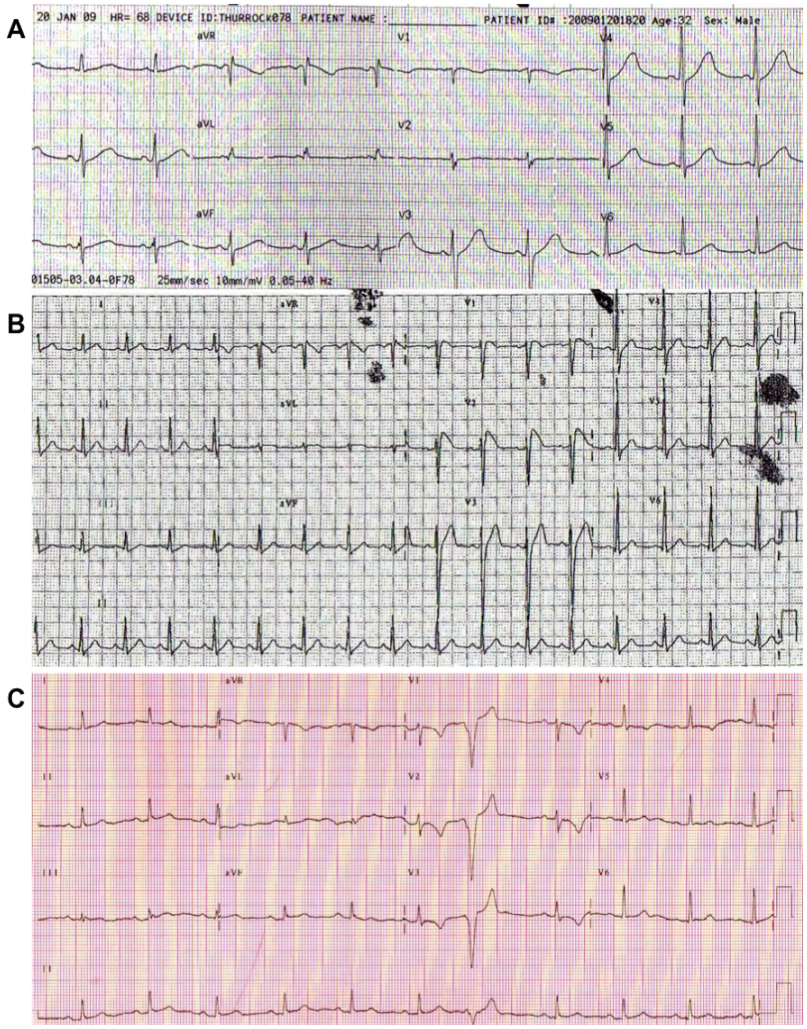


Figure 11: Examples of diagnostic ECGs of SADS victims obtained prior to the fatal cardiac arrest. None of the victims had received the diagnosis suggested by the ECG. A. ECG demonstrating a long-QT interval (QTc: 500 msec) obtained by ambulance paramedics in a 32-year-old male with recurrent episodes of epileptic fits on treatment with Levetiracetam (Table 11); B. ECG demonstrating the type-1 Brugada phenotype performed during a hospital admission for viral gastroenteritis in a 20-year-old male. The victim was pyrexial during the admission. He had never reported any cardiovascular symptoms; C. ECG demonstrating anterior T-wave inversion and a ventricular ectopic beat of LBBB morphology in a 16-year-old female. The ECG was obtained during cardiovascular evaluation for episodes of palpitations and pre-syncope. The echocardiogram was reported as normal. A 24-hour Holter monitor demonstrated >1,000 ventricular extrasystoles (Table 11).

4.5.2 Characteristics of family relatives evaluated

The great majority (n=227; 84%) of individuals evaluated were 1st-degree relatives of the deceased. In only 29 out of the 82 families (36%) we had the opportunity to evaluate both natural parents of the deceased. This was predominantly due to a considerable number of parents being divorced or separated, or as a result of the fact that parents were not keen to be investigated themselves as long as their offspring was evaluated, particularly in cases where the parents did not have siblings with children that could have potentially inherited a condition predisposing to SADS. In a small number of families (n=6) where the deceased was of an older age (>45 years), parents were either deceased or of advanced age. Nineteen families (23%) comprised of only one relative who was referred for evaluation.

Of the 271 relatives evaluated, 225 (83%) completed the whole array of investigations that were considered necessary by the investigators, based on the family history and initial findings in the individual and his/her relatives. The majority of individuals that did not complete the evaluation did so because of a young age (<16 years). All children were subjected to a resting ECG and those over the age of 8 years also underwent a transthoracic echocardiogram. All children were followed-up in our clinic with a view to receive all necessary investigations when they attain age of 16 years. Children with symptoms or family history causing concern were referred for comprehensive evaluation by a paediatric cardiologist at the Royal Brompton hospital and St George's hospital.

Fifty-three of the family members (19%) had experienced cardiovascular symptoms including syncope (n=26), palpitations (n=22), dizzy spells/pre-syncope (n=19), chest pain (n=14) and shortness of breath (n=3). Half of the symptomatic individuals (n=27), including

19 of the 26 individuals who had experienced syncope, had sought medical attention. Syncope had been attributed to vaso-vagal syncope (n=8), vertigo (n=2), primary epilepsy (n=1), epileptic fit due to intracranial bleed (n=1), migraine attack (n=1). In the other 6 cases no diagnosis was reached.

Table 12: Family relatives characteristics	
Total number of relatives evaluated	271
Number of relatives evaluated/family	3.33
Relatives who underwent full investigations	225
Reasons for not completing full complement of investigations: Young age Refused investigations Did not attend Co-morbidities Pregnancy	 25 9 8 2 2
Mean age [range] (years)	34 [1-78]
Gender (male)	129 (48%)
Prior cardiovascular symptoms: Syncope	53 (19%) 26 (10%)

4.5.3 Results of clinical evaluation

Of the 83 families evaluated in our clinics, 40 (48%) were diagnosed with a definite or probable/possible inherited cardiac disease based on the results of the comprehensive cardiac evaluation (Figure 12). In the majority of families diagnosed with a condition (34 out of 40 families, 85%), an inherited arrhythmogenic syndrome was identified. Brugada syndrome (n=26) and BrS related overlap syndromes (n=4) accounted for 74% of the conditions identified. The remaining diagnoses were LQTS (n=4), DCM (n=2), ARVC (n=2), familial AF (n=1) and coronary artery spasm (n=1).

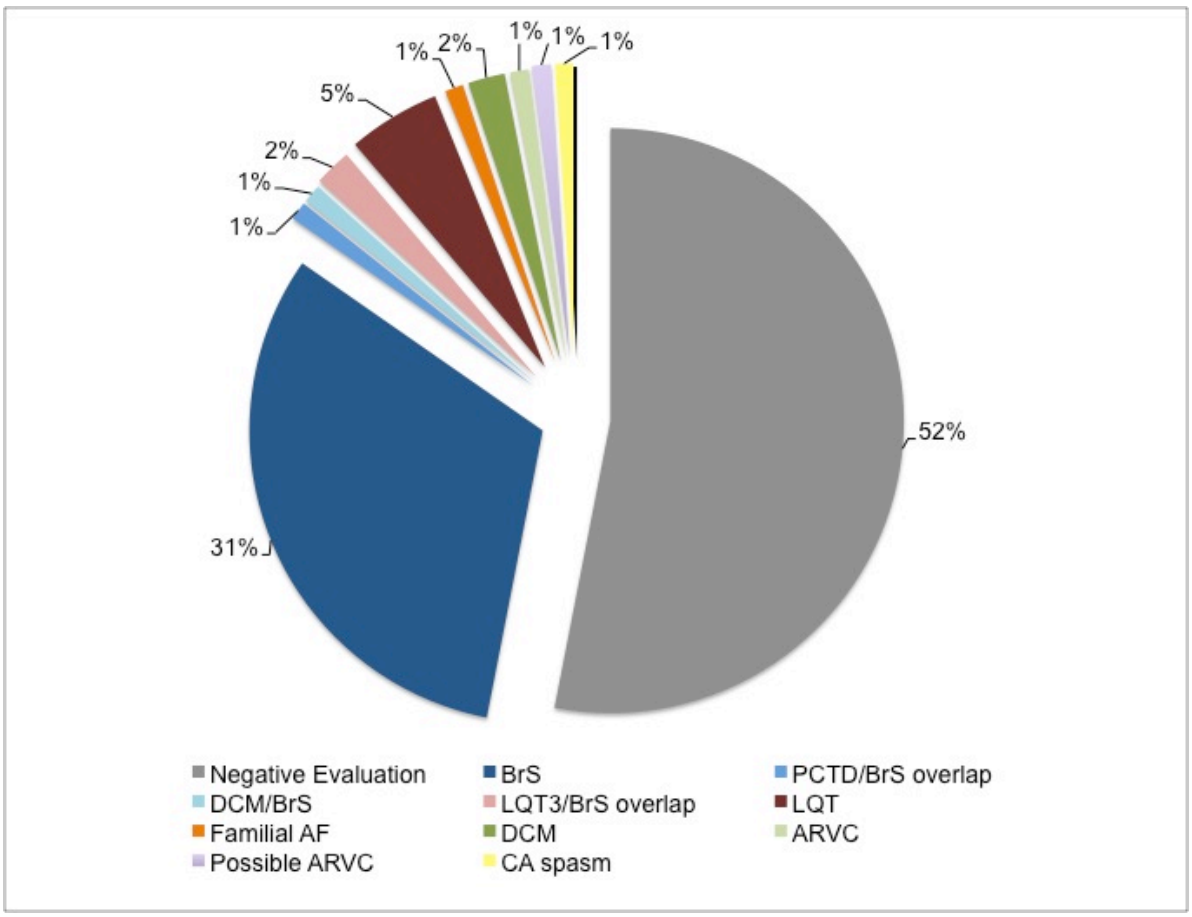


Figure 12: Pie chart depicting the diagnoses reached in SADS families as a result of the comprehensive cardiac evaluation of surviving relatives. Results are expressed as percentage (%) of the total number of families included in the study.

AF: Atrial fibrillation; ARVC: Arrhythmogenic right ventricular cardiomyopathy; BrS: Brugada syndrome; CA: Coronary artery; DCM: Dilated cardiomyopathy; LQT3: Long QT syndrome type-3; PCTD: Premature conductive tissue disease.

Of the 271 relatives who attended our clinic, 65 (24%) were diagnosed with a definite or probable/possible cardiovascular pathology, which had not been previously identified. Fifty-three relatives were diagnosed with BrS or a BrS overlap syndrome, 7 relatives received a diagnosis of LQTS, 3 individuals were labeled with a diagnosis of familial DCM, 1 individual was thought to exhibit signs of possible ARVC and 1 was diagnosed with familial AF.

4.5.4 Predictors of a positive diagnosis

The authors tested a number of variables to identify predictors of a positive diagnosis on a family (Table 13) and an individual (Table 14) level. On a family level, families who received a diagnosis had a higher mean number of relatives assessed compared to families with no diagnosis, but the difference was not statistically significant. On an individual level, the presence of syncope in family relatives appeared to correlate with an underlying cardiac pathology. Moreover, all of the individuals who received a diagnosis were subjected to the entire complement of investigations as outlined in figure 10. On the contrary only 78% of individuals with no diagnosis were subjected to all necessary investigations. Both groups were otherwise of similar characteristics.

Table 13: Predictors of diagnosis of inherited heart disease in evaluated families.

	Positive diagnosis (n=40)	**No diagnosis (n=43)**	**p-value**
No of relatives evaluated	3.9±3.1	2.8±1.7	p=0.056
Both parents of victim evaluated	14 (35%)	15 (35%)	p=1.000
Age (yrs)	29.0±12.2	28.8±10.8	p=0.950
Male gender	27 (68%)	31 (72%)	p=0.637
FH of SCD	10 (25%)	9 (21%)	p=0.794
Mode of death (at rest/asleep)	32 (80%)	26 (60%)	p=0.154

Table 14: Predictors of diagnosis of inherited heart disease in evaluated relatives.			
	Positive diagnosis (n=65)	**No diagnosis (n=206)**	**p-value**
Age (yrs)	37.3±14.5	33.3±17.8	p=0.110
Male gender	28 (44%)	101 (49%)	p=0.472
Cardiac symptoms	18 (28%)	35 (17%)	p=0.100
Syncope	12 (19%)	14 (7%)	p=0.007
Completed all necessary investigations	65 (100%)	161 (78%)	p<0.001

4.5.5 Families with a positive diagnosis

Brugada syndrome

Twenty-six families, comprised of 108 relatives, received a diagnosis of BrS based on the identification of the type-1 Brugada phenotype in 43 relatives evaluated in our clinic. Of note, 16 relatives did not complete all necessary investigations and in particular they were not subjected to an Ajmaline provocation test, primarily due to young age at the time of the evaluation. As a precaution, all 16 individuals were offered the conventional life-style modification advice and regular follow-up in our clinic. We aim to perform the Ajmaline provocation tests once individuals reach the age of 16 years, unless they present with new onset of sinister symptoms, suggestive of malignant arrhythmias.

Two families, comprised of 10 individuals, were diagnosed with a LQT-3/Brugada phenotype. The first family was referred to our clinic after the death of a 29-year old male while driving. His father had died suddenly at the age of 52 years and was attributed to “heart attack” and there was a family history of 2 sudden infant death syndrome (SIDS)

deaths at 3 and 10 months. One of the deceased's sisters was under investigations for recurrent episodes of syncope. Six of the 8 family members evaluated exhibited a positive Ajmaline provocation test, of whom 3 also demonstrated a long QT interval with the typical LQT-3 phenotype (long-ST segment with small T-wave). The second family, was comprised of 2 brothers who were referred after their mother experienced a fatal cardiac arrest at the age of 41 years. She had previously reported episodes of palpitations and pre-syncope. Her father died suddenly at the age of 53 years and there was another sudden death in a paternal aunt. No further details were available given that the family was originally from south Italy. One of the brothers had an LQT-3 pattern on his 12-lead ECG and a positive Ajmaline provocation test.

One family, comprised of 4 individuals, was diagnosed with a DCM/Brugada overlap phenotype. The family was referred to our clinic after the death of the son at the age of 19 years. He had never reported any cardiovascular symptoms. He died in his sleep and subsequent post-mortem evaluation by our cardiac pathologist revealed a normal heart. Cardiac evaluation identified LBBB with QRS duration of 150 msec and moderate left ventricular systolic dysfunction in his mother. There was no evidence of scarring on the CMR. Due to the considerable QRS prolongation she was not subjected to an Ajmaline test. One of the deceased's half-brothers (maternal) exhibited the Brugada phenotype during Ajmaline testing but imaging studies were unremarkable.

A 27-year-old male was diagnosed with progressive conductive tissue disease/Brugada overlap. He was referred to our clinic after the death of his brother. The rest of the family lived in India. Review of the family history revealed that 2 of their brothers had died at a young age (45 years, 48 years) and 2 other siblings had been implanted with a permanent pacemaker for recurrent episodes of syncope at a young age (<35 years). The patient

reported episodes of pre-syncope and syncope. His 12-lead ECG demonstrated sinus rhythm with a normal PR and QRS interval. An Ajmaline provocation test identified the Brugada phenotype. On a Holter monitor the patient exhibited intermittent Mobitz type-2 (2 to 1) second degree heart block and complete heart block.

Exercise testing in Brugada syndrome

(Case report publication attached in appendix 3)

Of the 108 relatives evaluated in families with a diagnosis of BrS or BrS overlap syndromes, 85 were subjected to an exercise treadmill test, including 43 individuals with an established diagnosis. Of these 43 individuals, 3 (7%) developed the diagnostic, type-1 Brugada phenotype, predominantly during the recovery phase.[247] Of interest, in contrast to reports in the literature which describe normalisation of the ST-segment elevation, with increased sympathetic activity during exercise and unmasking of the Brugada phenotype with increased parasympathetic tone in recovery, one of the individuals developed the Brugada phenotype during peak exertion, refuting this simplistic theory as the sole explanation (Figure 13). Moreover, one individual developed multiple, monomorphic ventricular ectopic beats of left bundle branch block pattern with inferior axis indicating a right ventricular outflow tract origin. The ectopic beats were first noted at the beginning of recovery and subsided quickly (Figure 14). We had the opportunity to repeat exercise testing in 2 out of the 3 individuals and they consistently developed the Brugada pattern and ventricular ectopy.

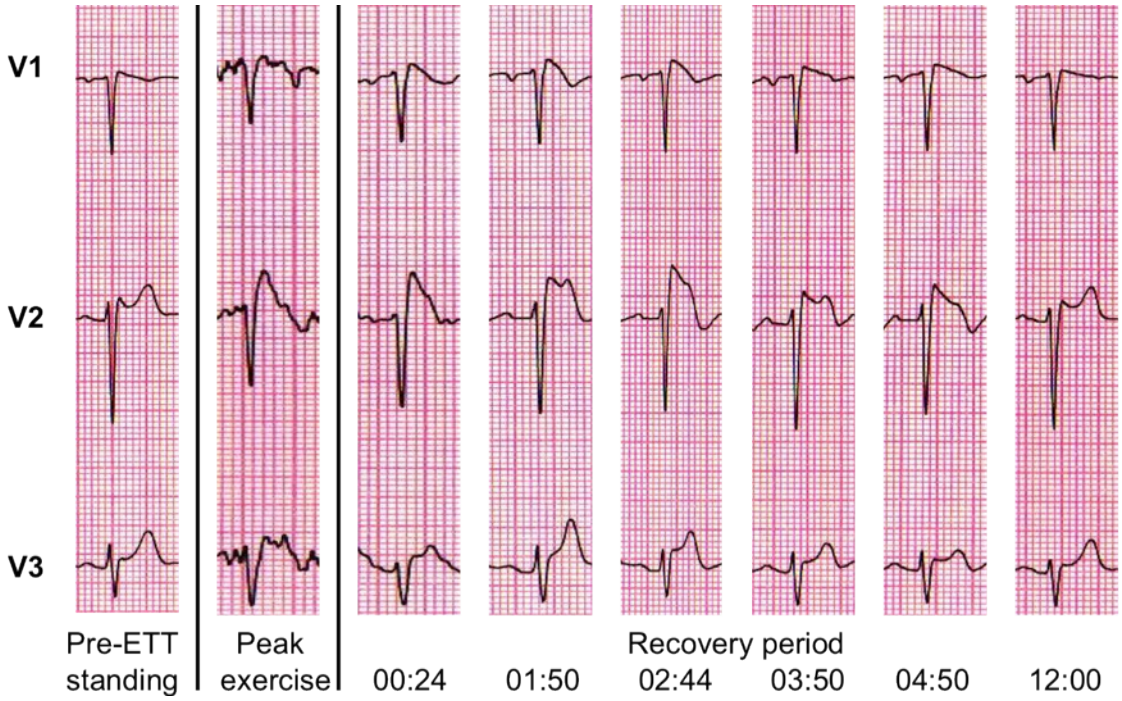


Figure 13: ECG strips of leads V1–V3. ST-segment changes before, at peak and during the recovery phase of the treadmill exercise stress test. ETT, exercise tolerance test

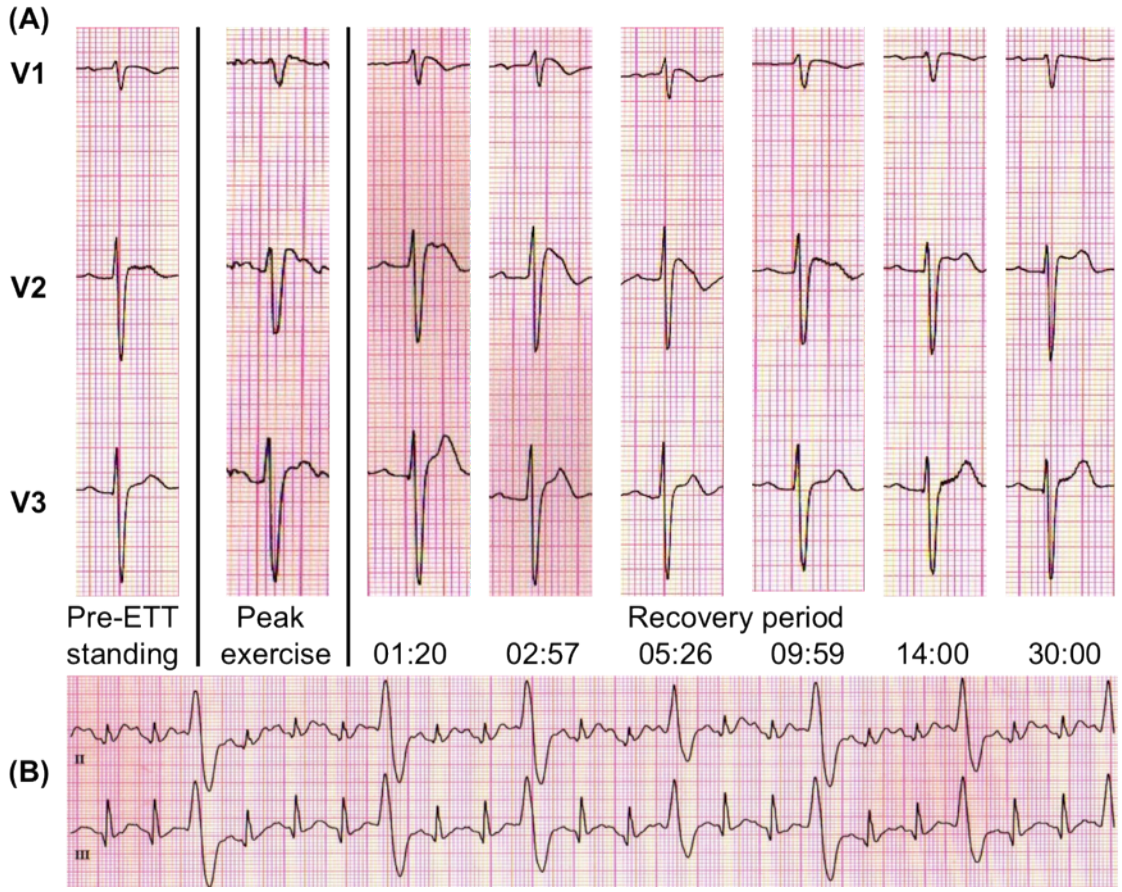


Figure 14: (A) ECG strips of leads V1–V3. ST-segment changes before, at peak and during the recovery phase of the treadmill exercise stress test. (B) Rhythm strip demonstrating multiple ventricular extrasystoles of increasing frequency 40 sec into recovery. The ectopics terminated spontaneously within 10 sec. ETT, exercise tolerance test.

Long-QT syndrome

Four families, comprised of 15 individuals, received a diagnosis of LQTS. The first family was referred to our clinic after the death of a 17-year-old female when the alarm clock went off. She had previously reported episodes of palpitations and dizzy spells and in retrospect a 12-lead ECG performed by her GP exhibited bifid T-waves with a QTc duration of 470 msec, consistent with an LQT-2 syndrome. There was also a family history

of sudden death in her maternal aunt. On familial evaluation both the deceased's mother and her cousin (daughter of the maternal aunt) were diagnosed with LQTS. The second family was referred after the death of their 17-year-old son during sleep. His mother and 3 of his sisters exhibited a prolonged QT interval (QTc: 481-533 msec) associated with paradoxical prolongation of the QT interval during exercise testing. The third family was diagnosed with LQTS based on the presence of a QTc of 490 msec in the mother of a 26-year-old female that died at rest, with no prior symptoms or sinister family history. Finally, the diagnosis of LQTS in the fourth family relied solely on the review of the family history and the 12-lead ECG of the deceased (Figure 11A and Table 11). The father and the deceased's paternal half-brother and half-sister (from the father's second marriage) were referred for evaluation after the death of a 32-year-old male who had experienced several episodes of epileptic seizures and was on treatment with Levetiracetam under the care of a neurologist. The ECG obtained during one of his hospital admissions exhibited a corrected QT-interval of 550 msec. Moreover, review of the family history revealed that the deceased's mother (father's first wife) had also died suddenly at the age of 33 years, as had his maternal grand-mother (early thirties). As expected, clinical evaluation of the father and paternal half-siblings of the deceased was negative, given that they were not genetically related to the maternal line of the victim.

Dilated cardiomyopathy

Two families, comprised of 8 individuals, were diagnosed with familial DCM. In the first family the diagnosis was based on the identification of LBBB on the 12-lead ECG with associated dilated LV cavity (LVIDd: 62 mm), moderate left ventricular systolic dysfunction and global hypokinesia in the sister of the deceased. Due to her age (56 years) a CT coronary angiogram was performed which revealed only mild atheroma of the left anterior

descending coronary artery. In the second family the diagnosis of DCM was based on the identification of a dilated LV cavity with mild LV systolic dysfunction in the father and brother of the deceased.

Arrhythmogenic right ventricular cardiomyopathy

Two families were diagnosed with possible ARVC. In the first case the diagnosis was suspected after review of investigations of the deceased. Her post-mortem, including slides reviewed by our cardiac pathologist, was negative. The deceased was a 16-year-old female who had been investigated for episodes of palpitations and pre-syncope. Her 12-lead ECG (Figure 11C) demonstrated T-wave inversion in leads V1-V3 with associated ectopy of LBBB morphology. Her 24-hour Holter monitor demonstrated >1,000 ventricular extrasystoles. An echocardiogram had been reported as normal. Evaluation of her mother was negative. In the second case the daughter of a 40-year-old man was referred for evaluation after he was found dead in his bed. The daughter's ECG revealed T-wave inversions in leads V1-V5 but ECHO and CMR did not reveal any convincing evidence of cardiomyopathy. She subsequently developed one episode of syncope but had no documented episodes of arrhythmia. She is currently under regular review.

Familial atrial fibrillation

One family was diagnosed with familial AF. A 36-year-old female died in her sleep. She had a past medical history of paroxysmal AF and was on beta-blockers as and when required. Her 46-year-old sister was noted to be in atrial flutter during her evaluation in our clinic. A Holter monitor revealed sinus rhythm with frequent episodes of atrial fibrillation

and atrial flutter. An echocardiogram and CMR were normal. An Ajmaline test was negative.

Coronary artery spasm

The diagnosis of coronary artery spasm was based on the review of the hospital records of a 31-year-old male whose family was referred to our clinic after he died suddenly at home. The deceased 5 years prior to his death had been admitted with VF arrest, preceded by chest pain. His ECG demonstrated ST-segment elevation in the lateral leads and he was thrombolysed. A subsequent coronary angiogram did not identify any significant coronary artery atheroma and an echocardiogram was normal. There was a very mild Troponin rise. He was commenced on treatment with calcium channel blockers. An ICD was not deemed necessary based on the fact that VF arrest had occurred in the context of ischaemia due to coronary artery spasm. The post-mortem was performed by our cardiac pathologist and failed to identify any significant pathology and in particular macroscopic or microscopic findings consistent with coronary artery spasm (Table 5). Alternative diagnoses such as an acute embolic event or acute myocarditis may have accounted for the initial presentation and subsequent death. Comprehensive evaluation of the deceased's mother, brother and maternal half-sister was negative.

4.5.6 Families with no diagnosis

In almost a third of the families (15 out of 43) where no diagnosis was established, one or more relatives did not complete all necessary investigations. The majority of them did not undergo an Ajmaline provocation test. As a result a diagnosis of BrS may have been missed in a considerable proportion of families.

4.5.7 Further evaluation

Only a small number of relatives evaluated in our clinic required further investigations. Those included computer tomographic coronary angiography (CTCA) (n=7) and conventional coronary angiography (n=2) to exclude coronary artery disease and one case with negative clinical evaluation and recurrent episodes of vaso-vagal sounding syncopal episodes who was referred for a tilt-table test and neurological evaluation. Electrophysiological studies were performed in only 3 cases; in one case of an individual with negative familial evaluation who was reporting episodes of palpitations and associated syncope and in two cases for inducible VT in order to offer further reassurance after both were diagnosed with the Brugada phenotype on Ajmaline provocation testing.

4.5.8 Mutation analysis

We performed mutation analysis in 21 out of the potential 38 families with phenotypes suggestive of inherited cardiac conditions. Of the 17 families with a positive phenotype but no mutation analysis: 10 BrS families genetic testing was either referred to their local geneticist or is still pending in our genetics laboratory due to funding restrictions; 3 families (2:BrS, 1:possible ARVC), individuals declined genetic testing after counselling; 1 family with a diagnosis of LQTS and 1 family with a diagnosis of possible ARVC no genetic testing was performed since the diagnosis was based on review of the deceased's ECG, family history and past medical history but the phenotype was absent in evaluated relatives; 2 families diagnosed with DCM mutation analysis was not performed due to the absence of co-existing atrio-ventricular block.[214]

Of the 30 families with a diagnosis of BrS and BrS related overlap syndromes, we performed genetic testing in 18 families. Four families had *SCN5A* mutations thought highly likely to be disease causing (*E1784K*; n=2, *D349H*; n=1, *Q1112X*; n=1). The *E1784K* mutation in the *SCN5A* gene has been described in the literature and is associated with BrS, LQTS and premature conduction tissue disease. In agreement with existing literature, the 2 families with an *E1784K* mutation expressed a LQT-3/BrS overlapping phenotype.[204,207,233,248,249] *D349H* is a novel mutation, which affects a highly conserved residue (R349) located in the first pore segment of the *SCN5A* channel, at the loop between segments 5 and 6, where several missense mutations have been associated with sodium channel "loss-of-function", consistent with BrS. Finally, *Q1112X* is a stop mutation in exon 18 of the *SCN5A* gene resulting in truncation of protein and predicted nonsense mediated decay, which in general are related to disease.

In one family an *I1377V* mutation of *SCN5A was detected*. This is a novel heterozygous missense variant that is rare and absent from over 12,000 alleles in the multi-ethnic NHLBI Exome Variant Server. The variant affects a moderately conserved residue (*I1377*), producing a change from non-polar isoleucine to non-polar valine. This change is predicted to produce a small change in the mass (Grantham Distance: 29 [0-215]). The amino acid I1377 is located in the loop between the segments 5 and 6 of transmembrane domain 3 of the alpha subunit that forms the sodium channel. We performed in silico analysis: both Polyphen-2 and SIFT predict a benign protein effect. In contrast, topological analysis, which is recognised as superior to non-specific in-silico models in the long QT syndrome, would suggest that 88% of missense variants in this region associate with functional ion channel disease.[250]

Pathogenic mutations were identified in the two of the 3 LQTS families tested. A *G168R* mutation was identified in the *KCNQ1* gene. The mutation affects a highly conserved residue and has been extensively described as a cause of LQTS.[251-254] In the second family we identified a *A561V* mutation in *HERG*. The mutation results in substitution of valine for a highly conserved alanine at codon 561, altering the S5 transmembrane segment of the *KCNE2* encoded protein.[218,255,256]

4.5.9 Immediate management

All of the 65 relatives who received a definite or probable/possible diagnosis of an inherited cardiac condition, received the necessary life-style modification advice.[161,162,164,165,257-265] Life style modification advice was also offered to a small number (n=2) of individuals with inconclusive investigations who were not labelled with a diagnosis and individuals with a familial diagnosis who did not complete the investigations (n=16), predominantly due to young age. Of the 271 relatives evaluated 26 (10%) received some from of medical intervention. Medical therapy was initiated in 11 individuals; 7 individuals diagnosed with LQTS received prophylactic therapy with a beta-blocker and 4 individuals with DCM were commenced on treatment with a beta-blocker and an angiotensin converting enzyme inhibitor (ACE-i). Fifteen individuals, all with a diagnosis of BrS (n=8) or BrS/LQT-3 (n=7) overlap syndrome were implanted with an ICD. Five individuals with BrS were considered to be at high risk of sudden death based on the presence of previous unheralded syncope. In two individuals an ICD was implanted based on an inducible type-1 Brugada pattern during the recovery phase of the exercise treadmill test. Although there is no established association between unmasking of the Brugada phenotype during exercise and increased risk of SADS, some studies have reported ST-elevation during exercise testing as a predictor of prognosis.[159,258] One 17-year-old female who did not

exhibit any of the conventional risk factors opted for an ICD after detailed discussion relating to the uncertainties of risk stratification in BrS and the potential complications of an ICD implantation. Of the 7 relatives with a familial diagnosis of BrS/LQT-3 overlap syndrome, we recommended ICD implantation in all of them, based on evidence indicating that such individuals are at excess risk of sudden death.[53,193,259,260] One exhibited episodes of unheralded syncope and two others had exhibited a number of syncopal episodes, which were associated with prodromal symptoms and probably represented vaso-vagal syncope. Finally, the one individual with evidence of frequent episodes of paroxysmal Atrial Fibrillation/Atrial flutter was initially commenced on anticoagulation therapy with Warfarin and was subsequently offered an AF ablation, which was judged to be successful on initial follow-up.

4.5.10 Short-term follow-up study

We were able to obtain follow-up data either through subsequent follow-up in our clinic or from an audit study in 55% of the families included in our SADS study. Data were available on 30 out of the 40 (75%) families where a diagnosis was identified after familial evaluation, but only in 16 out of the 43 (37%) families where no pathology was identified (p=0.001). The mean follow-up was 25.5±16.8 months versus 19.1±12.5 months (p=0.188) for families with a diagnosis compared to the ones without a diagnosis, respectively.

The 30 families diagnosed with a condition comprised of 119 relatives. The respective follow-up period equated to 244 person-years. During that period there was an appropriate ICD discharge, one unexpected death and a new diagnosis of BrS. The death occurred in a family with a diagnosis of BrS where 4 of the deceased's relatives had been investigated, namely the deceased's mother, sister and two maternal half-siblings. The

mother and the half-brother had been diagnosed with BrS based on the presence of the Brugada phenotype during Ajmaline provocation test. The sudden death occurred in the sister of the proband who initiated the familial evaluation. She died in her sleep at the age of 37 years, 6 years after her initial evaluation in our clinic. A subsequent post-mortem evaluation of the heart by our specialist cardiac pathologist failed to identify any underlying cardiac abnormality and, therefore, by definition she was a victim of SADS. Review of her clinic evaluation and further discussion with her parents, revealed that the victim had never experienced any symptoms and all investigations including an Ajmaline provocation test had been normal, indicating a potential false negative Ajmaline provocation test. The half-sister of both victims of SADS that had been cleared in our clinic after negative Ajmaline provocation testing also received a diagnosis of BrS after repeat Ajmaline testing at her local centre and was implanted with an ICD.

Of the 15 patients implanted with a defibrillator, during a follow-up period of 31.6 person-years one individual received an appropriate treatment and two developed complications during initial implantation of the device. The ICD discharge occurred in an individual diagnosed with BrS who was offered an ICD as a result of having experienced syncope. On review of the ICD traces the patient initially developed polymorphic VT, which disintegrated to VF. Sinus rhythm was restored after a single shock. Of the two individuals who experienced early complications, one developed right ventricular perforation with tamponade at the time of implantation and required lead repositioning and the second experienced multiple inappropriate shocks within 24 hours of implantation due to lead displacement, which also required repositioning.

No events or new diagnosis occurred in the 16 families with no diagnosis, comprised of 59 relatives. The total follow-up period was 88 person-years.

4.6 Discussion

This study is one of the largest, prospective studies investigating SADS deaths. In contrast to most of the existing studies, referral of all families included in our study was triggered by a "true" SADS death, as evident by the post-mortem report, and most importantly all relatives were evaluated in a single centre, under the same team and largely with the same screening protocol. Results from the comprehensive familial cardiovascular evaluation in our inherited cardiac diseases clinics indicate that in almost 50% of SADS families an underlying cardiac pathology was detected, allowing for a probable cause of death to be identified but most importantly for individuals at potential risk of sudden death to be recognised. Nearly a quarter (24%) of family relatives assessed were diagnosed with a previously unsuspected cardiac pathology and managed according to established protocols in an attempt to minimise their risk of SADS. That percentage rises to 29% if we consider only relatives who underwent comprehensive cardiovascular investigations, since almost a fifth (17%) of individuals attending our clinic had limited screening, predominantly as a result of their young age.

Our study suggests that some of the SADS deaths may have been preventable, since more than a third (37%) of SADS victims had reported cardiovascular symptoms prior to their death and 17% had experienced at least one episode of syncope, which is likely to be considered a sinister symptom and trigger medical consultation. Unfortunately in a small number of cases (n=5) the syncope and seizures had triggered a neurology referral and the deceased had been diagnosed with epilepsy and possible transient ischaemic attack. In two cases the 12-lead ECG was available and demonstrated features that should have raised suspicion on underlying primary arrhythmogenic syndrome, namely a prolonged QT interval and a type-2 Brugada pattern (Table 11 and Figure 11A). Furthermore, almost a

quarter of victims had a family history of premature sudden death, with 12% involving a first-degree family relative. Based on the 8th chapter on arrhythmias and SCD of the National Service Framework for heart disease, which has been in place since 2005, such deaths should trigger a referral for specialist review in an attempt to prevent further tragedies.[8]

4.6.1 Comparison of the thesis results with existing studies

The diagnostic yield of cardiac pathology in 48% of SADS families in our study is in keeping with existing studies, although direct comparisons are not always possible due to the different definitions and methodology used. The Netherlands group published two studies in individuals with a sudden unexplained death, but not necessarily SADS.[81,87] They reported a diagnostic yield of familial evaluation of up to 40% with primary arrhythmogenic syndromes accounting for the majority of the diagnoses (31 of 47; 66%). Although in their initial cohort the predominant diagnosis was CPVT, in the second, larger cohort LQTS (n=10), BrS (n=9) and CPVT (n=8) were equally represented. As expected, given that the inclusion criteria did not require a normal post-mortem, a diagnosis of structural heart disease accounted for 34% of the conditions identified compared to only 13% in our cohort. The study by Caldwell et al[89] reported a lower diagnostic yield of 30% but only a limited number of relatives received comprehensive screening similar to our study. Finally, the study by Behr et al.[82] is the one that most closely resembles the inclusion criteria and methodology employed in our study. Behr et al. reported a diagnostic yield of 53% with the predominant diagnosis being LQTS. In contrast to our study where molecular autopsy was not performed, the study by Behr et al. utilised molecular autopsy alongside clinical evaluation, which is likely to have enhanced to some extent their diagnostic yield. The authors performed molecular autopsy in 24 victims, 5 of whom

identified a pathogenic mutation. In one case the familial diagnosis was based on the molecular autopsy result alone, while in the other 4 cases the interpretation of the clinical findings are likely to have been affected by the knowledge of a positive mutation. Similarly to the Netherlands group cardiomyopathies accounted for a considerable proportion (30%) of the established diagnoses, despite the strict inclusion criteria of victims with a normal post-mortem alone.

4.6.2 Brugada syndrome

In our study the predominant familial diagnosis was of BrS or BrS related overlap syndromes, which accounted for 75% of the diagnostic yield and 36% of the overall SADS deaths. On an individual level, 53 out of the 65 relatives (82%) who were diagnosed with a definite or probable/possible cardiovascular pathology exhibited the Brugada phenotype. This is in contrast to existing studies where BrS formed a relatively small proportion of the diagnostic yield and other primary arrhythmogenic syndromes such as LQTS and CPVT predominated. In the studies by the Netherlands group BrS was identified as a potential cause of sudden death in only 6%-7% of families,[81,87] while the UK studies by Behr et al.[82] and Caldwell et al.[89] reported a diagnostic yield of BrS of 9% and 2%, respectively. There are several factors that may account for the predominance of BrS in our cohort including differences in the baseline cohort characteristics, the frequency of Ajmaline provocation testing and most importantly the use of the higher intercostal V1 and V2 ECG leads.

Activity levels at death

The great majority of the SADS victims in our study (70%) died at rest, with 40% dying in their sleep. Brugada syndrome is strongly associated with sudden death at rest and in

particular during sleep due to increased vagal tone that appears to play an important role in inducing ventricular arrhythmias. In contrast most other relatively prevalent primary arrhythmogenic syndromes such as LQT-1, LQT-2 and CPVT and most cardiomyopathies are predominantly linked to deaths during exertion or emotional stress. In the initial study by the Netherlands group only 37% of sudden death victims died at rest or in their sleep, with 44% dying on exertion, which was reflected in the pathologies identified (CPVT, LQTS, HCM, ARVC). In the second, larger study by the same group the proportion of individuals who died at rest or in their sleep increased to 62% while those dying on exertion reduced to 31%, which was reflected in the pathologies identified with BrS accounting for a similar proportion of deaths to LQTS and CPVT. A similar proportion of individuals died at rest in our study (70%) and the study by Behr et al. (63%) indicating that other potential contributors may have influenced the diagnostic yield differences between the two studies.

Ajmaline provocation test

One fundamental difference in our methodology compared to most of the existing studies is the frequency of performing provocation testing with class-1 anti-arrhythmics to unmask the Brugada phenotype. It is well established that the baseline ECG is of limited diagnostic value given that in the majority of Brugada patients it may be normal or fluctuate between normal and the Brugada pattern.[158,168,169] This is further reinforced by our study where only 2 individuals exhibited an unequivocal type-1 ECG pattern in the absence of Ajmaline. In our SADS cohort 206 out of the 271 relatives (76%) evaluated were subjected to an Ajmaline provocation test. The great majority of individuals underwent Ajmaline testing because of a normal ECG and echocardiogram and the absence of an alternative diagnosis. In contrast to our study, the Netherlands group performed only a limited number

of provocation tests with class-1 anti-arrhythmics in individuals “when BrS was suspected”, without specifying what were the exact characteristics that raised suspicion of BrS. In the study by Caldwell et al. only 20 out of the 193 (10%) individuals were subjected to Ajmaline provocation testing based on “a history and/or ECG suggestive of BrS”.

Utilizing the higher intercostal leads

Detailed discussion of the utility and implications of higher intercostal leads is included in the discussion of chapter 6. Based on the results form the familial evaluation of SADS victims, the use of higher intercostal leads more than doubled our diagnostic yield of BrS. Performing ECG with leads V1 and V2 placed in the 4^{th} IS, only, would have missed the diagnosis in 7 out of the 26 Brugada families of our SADS cohort reducing the diagnostic yield of BrS to 23%. The overall diagnostic yield would drop from 48% to 40%, which is more in keeping with that of the Netherlands group and Caldwell et al. Additionally, we would have failed to identify 18 out of the 43 individuals diagnosed with BrS, reducing the overall yield on an individual level from 24% to 17%.

Exercise testing in Brugada syndrome

Both the American Heart Association and the European Society of Cardiology recommend disqualification of Brugada patients from all competitive sports with a potential exception only for low-dynamic and low-static sports. Most experts however, view these recommendations as an overcautious approach, since there is no clear association between exercise and sudden death in BrS. In addition, exercise testing is not routinely used for the evaluation of Brugada patients or suspected carriers. In our cohort however, almost 15% of Brugada victims died during or shortly after exertion and almost 10% of the

family relatives diagnosed as carriers of the Brugada gene developed the Brugada phenotype at peak or immediately post exertion, which in two individuals was associated with brief episodes of multiple ventricular extrasystoles. These cases highlight the need to assess the role of exercise testing in the diagnosis and risk stratification of BrS and infer that BrS should be considered in the differential diagnosis in athletes presenting with syncope. This is of particular relevance since most physicians would consider syncope occurring immediately post exertion of fairly innocent nature, most probably representing a form of vasovagal syncope.

4.6.3 Short-term follow-up study

Due to the small number of events and the relatively short follow-up period we are unable to draw any safe conclusions form our follow-up study. Our data however, raise important issues that worth mentioning. The individual that tragically passed away despite being reassured after comprehensive evaluation was part of a family who received a diagnosis of BrS after identifying the diagnostic phenotype on Ajmaline provocation testing in the mother and the half-brother of the deceased. Her post-mortem by our expert cardiac pathologist was negative and as such she was by definition a victim of SADS. At repeat evaluation by another specialist after the second death, the half-sister that had also been reassured in our clinic, was diagnosed with BrS based on a positive repeat Ajmaline test. This particular family was screened in our clinic at the time when we utilized the conventional V1 and V2 leads, alone, which may account for the “false negative” results. Based on the results of chapter 5, evaluating the impact of higher intercostal leads, the diagnosis of BrS would be missed in half of the individuals and families if Ajmaline provocation testing were performed in the conventional leads only. There is also a paucity

of data relating to the sensitivity and specificity of Ajmaline testing with respect to gender and repeated tests.

This case also highlights the limitations of our risk stratification protocols in BrS, discussed in detail in chapter 6. This female victim did not exhibit any of the conventional risk factors, namely diagnostic ECG or symptoms suggestive of malignant arrhythmias. Based on current evidence, even if the Ajmaline provocation test had been positive, the patient would have been considered to be at low risk of SADS (around 0.5% per year) and as such would not have been offered an ICD. The risk of sudden death needs to be balanced against the potential risk of complications as a result of the ICD implantation. Current evidence suggests that the risk of ICD related complications, including inappropriate shocks, in Brugada patients can be up to 9% per year.[261,262,266] Inappropriate shocks are particularly relevant in individuals with BrS since they appear to be more prone to supraventricular tachyarrhythmias with reports of up to a fifth of the patients developing paroxysmal arrhythmias.[267,268]

4.6.4 Families with no diagnosis

In almost 50% of the families no diagnosis was established despite comprehensive clinical evaluation. Although no events or new diagnoses were noted during follow-up, given the limited number of families included and the short-term period, concerns still exist as to their potential risk of sudden death in these individuals. Potential explanations for the absence of any identifiable pathology include: 1. The pathogenic mutation was private to the victim of SADS either because it was a spontaneous mutation that the parents did not exhibit or because it was not passed down the family tree. Given that we did not perform molecular autopsy it would have been impossible to detect such mutations; 2. Our

evaluation failed to identify all affected relatives, as shown in one of the families diagnosed with BrS, where a new diagnosis of BrS in one of the relatives and a SADS death in another occurred, despite initial reassurance. This may be pertinent when one considers the impact of higher intercostal leads, given that in 44 out of the 261 (17%) individuals who underwent Ajmaline provocation in studies IV and V, the ECGs were performed with leads V1 and V2 placed in the conventional 4th IS only; 3. The death was secondary to idiopathic VF. In the study by the Netherlands group the investigators identified the risk locus at chromosome 7q36,[269] which is associated with familial idiopathic VF, in 3% of families;[87] 4. The death was due to a condition yet to be identified; and 5. The death was due to a non-cardiac cause that the post-mortem examination of the coronial pathologist failed to identify.

4.6.5 Limitations

The predominant diagnosis was of BrS. The authors concede that given the relative novelty of the condition and the considerable impact of the higher intercostal leads in the diagnostic yield, it is possible that some of the relatives exhibiting the Brugada phenotype did not have a genuine arrhythmogenic syndrome. However, all individuals who were diagnosed with BrS underwent comprehensive evaluation including a detailed echocardiogram and a significant proportion were subjected to CMR and none exhibited any evidence suggesting cardiac inflammation, ARVC or other structural cardiac anomalies. Further support for the presence of BrS is underscored by the genetic yield of pathogenic SCN5A mutations, which is similar to existing literature.[106]

The omission of regular epinephrine testing in individuals without an established diagnosis despite comprehensive cardiac screening may have underestimated the prevalence of

CPVT.[81] However, all relatives were subjected to maximal exercise testing and the superiority of epinephrine testing over exercise testing in inducing bidirectional arrhythmia and aiding a diagnosis of CPVT is still debated. Earlier studies suggested a higher sensitivity for epinephrine testing,[270] whereas a recent study indicated that exercise testing is of equal value, if not superior, to epinephrine testing and without the additional risk of inducing arrhythmias to non-affected family relatives.[271]

In the families in whom a pathogenic mutation was identified, we were unable to perform post-mortem analysis in the tissues of the victims for confirmation of the genotype since no tissue was available by the time the relatives were evaluated in our clinic. In the UK, the Human Tissue Act does not permit retention of tissue as part of a deceased patient's record, and retention for research requires familial consent at the time of post-mortem.[75] As such, in the majority of cases histological slides are prepared, reported and imaged at the time of the post-mortem examination, allowing early return of the tissue for burial or cremation.

Chapter 5: Familial evaluation of victims of sudden cardiac death with autopsy findings of uncertain significance

(Publication attached in appendix 3)

5.1 Introduction

Post-mortem evaluation of cases of SCD is an increasingly complex task and uncertainty may exist regarding the causal relationship between the pathological findings and the sudden death.[44] The significance of myxoid degeneration of the mitral valve with prolapse, stable atherosclerotic coronary plaque with limited (<50%) luminal stenosis and focal

myocarditis, which are relatively prevalent in the general population, may be erroneously overestimated. Not infrequently, post-mortem diagnosis of HCM is based on the presence of left ventricular hypertrophy (LVH) in the absence of myocardial disarray. Left ventricular hypertrophy however, is a recognized feature of physiological adaptation to exercise.[43,57] Similarly, despite the requirement for the presence of myocardial fibrosis on histological evaluation since the development of the original arrhythmogenic right ventricular cardiomyopathy (ARVC) criteria back in 1994,[83] commonly ARVC is still diagnosed solely on the presence of fatty infiltration of the right ventricular wall. Isolated fatty infiltration of the right ventricle however, is commonly present in obese individuals.[272] The distinction between pathology and normal variants may, therefore, be challenging in the context of SCD.

Overestimation of the significance of pathological findings can be associated with important consequences since the pathologist's report determines whether surviving relatives are referred for familial evaluation for potentially inherited cardiac conditions and more specifically whether subsequent investigations in surviving relatives are directed towards structural disorders or primary arrhythmogenic syndromes. This is reflected in the guidelines for autopsy investigation of SCD on behalf of the Association for European Cardiovascular Pathology, where the authors highlighted the concepts of "different degrees of certainty in defining the cause–effect relationship between the cardiovascular substrate and the sudden death event" and "grey zones".[44]

5.2 Aim

This study explored the hypothesis that a proportion of SCDs with autopsy findings of uncertain significance may represent part of the SADS spectrum, and in particular

inherited arrhythmogenic syndromes. The hypothesis was the product of the experience gained by the group's experience with screening family relatives after a SCD, which suggested that there was considerable variability in the interpretation of the causative effect of the pathological findings by non-cardiac pathologists, who tend to overestimate their significance, and accumulating evidence that individuals with arrhythmogenic syndromes attributed to genetic mutations may exhibit structural cardiac changes.

5.3 Personal contribution

The author performed prospectively the clinical evaluation of the 20 families assessed at Lewisham hosptial, including performing or supervising, analyzing and databasing the majority of investigations (ECG, echocardiography, exercise treadmill test, Ajmaline provocation test and Holter monitoring). Collected data on the deceased including data on previous admissions or GP consultations. The author reviewed and analysed all the data (form Lewisham and St George's hospitals) and drafted the published manuscript. The author was not involved with the post-mortem evaluation of any of the subjects that was performed by either the coroner's pathologist or our cardiac pathologist (MNS).

5.4 Methods

Study cohort

This study was performed in collaboration with an inherited cardiac disease clinic established at St George's Hospital since 2003. The clinic is lead by Dr E Behr, consultant electrophysiologist with expertise in conditions predisposing to SCD and SADS. Between 2003 and 2010, 41 families were referred to Lewisham hospital (n=20; see group 3 in

figure 9) and St George's hospital (n=21) cardiogenetics clinics, where the deceased's post-mortem revealed findings with structural abnormalities of uncertain causal effect as outlined in table 15. All deaths represented SCD from natural causes of apparently healthy individuals, as outlined in the inclusion criteria in the methods section of chapter 4. All victims had been subjected to a post-mortem where an extra-cardiac cause had been excluded and toxicology screen was negative.

Familial cardiological evaluation

The 41 families comprised of 157 blood relatives who underwent identical comprehensive cardiac evaluation according to the standard protocol for SADS families, as outlined in methods section of chapter 4 and figure 10. In the SADS cohort reported in chapter 4, CMR was performed only in relatives with ECG or echocardiographic features suggestive of cardiomyopathy. In this cohort with autopsy findings of uncertain significance all relatives diagnosed with an arrhythmogenic syndrome, where the deceased's post-mortem findings could be interpreted to represent a cardiomyopathy (LVH, myocardial fibrosis, ventricular dilatation and fatty infiltration of the myocardium) underwent CMR, even when the ECG or echocardiogram did not suggest structural heart disease. Further investigations were based on the overall clinical need.

Table 15: Pathological criteria classified of uncertain significance in sudden cardiac death autopsies (Group 3). For comparison with respective cardiac pathology associated with sudden cardiac death please refer to table 5.	
Macroscopic appearance	**Microscopic appearance**
Left Ventricular Hypertrophy NOT Hypertrophic cardiomyopathy	
Left ventricular wall thickness ≥15 mm and or heart weight ≥500g in males or ≥400g in females.	Myocyte hypertrophy with or without fibrosis. No myocyte disarray.*
Fatty infiltration NOT Arrhythmogenic right ventricular cardiomyopathy	
Fatty infiltration of the right ventricle.	Fatty infiltration of the right ventricular wall in the absence of fibrosis.
Dilated ventricles NOT Dilated cardiomyopathy	
Enlarged heart with heart weight ≥500g in males or ≥400g in females. Mild ventricular dilatation. No fibrosis.	Absence of interstitial fibrosis or myocardial inflammation.
Mild coronary artery disease NOT significant coronary artery pathology	
Atherosclerosis with estimated luminal narrowing ≤ 50% or 2mm probe patent in the absence of acute or chronic infarction	Absence of rupture/thrombosis of coronary artery and/or acute/chronic infarction in the LV
Scattered lymphocytic infiltrate NOT Myocarditis	
Normal	Scattered lymphocytic inflammatory foci with no fibrosis. No necrosis and/or degeneration of adjacent myocytes.
Mitral valve prolapse NOT Mitral valve rupture	
Floppy mitral valve with mild ballooning between chordae in one or both leaflets	Myxoid change in the valve
Bicuspid aortic valve NOT Aortic valve stenosis	
Two leaflets associated with no significant nodular calcification or aortic orifice stenosis.	Absence of left ventricular hypertrophy and fibrosis.

* isolated myocyte disarray confined to the anteroseptal and posteroseptal junctions should be considered normal

Ethical approval

Data collection was performed as part of the clinical evaluation of the families as proposed by the Government in the 8th chapter of the National Service Framework for Heart Disease, which includes guidelines for early identification of individuals at risk of sudden cardiac death and better support for families of victims.

Statistical analysis

Data interpretation and analyses were performed using R version 2.15.2 (R Development Core Team). Data were expressed in mean ± SD. Comparison of population proportions used Fisher exact test with Donner's adjustment as necessary to account for clustered data.

5.5 Results

5.5.1 Characteristics of cases of sudden cardiac death and blood relatives

The majority of cases of SCD were male (male to female ratio of 4:1). Most deaths (61%) occurred at rest or during sleep. A significant proportion (37%) had previously reported cardiac symptoms, and in 25% of cases there was prior family history of premature SCD (<50 years), none of which had prompted familial cardiac investigation (Table 16).

Table 16: Characteristics of victims of sudden cardiac death with autopsy findings of uncertain significance.	
Number of victims	41
Mean age [range] (years)	29.9±14.4 [4-59]
Gender (male)	80%
Ethnicity (Caucasian)	93%
Mode of death Asleep/At rest During/post exertion Unknown	 61% 37% 2%
Antecedent cardiac symptoms* Syncope	37% 15%
Prior family history of premature (<50 years) SCD	25%
Post-mortem review by expert cardiac pathologist	39%

* chest pain, palpitations, shortness of breath, pre-syncope, syncope

One hundred and fifty-seven blood relatives (48% male) were evaluated, with a mean age of 33.7±17.9 years, (range 9-70 years). Almost a quarter (23%) of the evaluated relatives reported cardiac symptoms with 10% having experienced at least one episode of syncope in the past.

5.5.2 Autopsy findings and results of familial evaluation

The post-mortem findings of uncertain aetiological significance in the 41 cases are illustrated in Figure 15. Following familial evaluation, 21 (51%) out of the 41 SCD cases were considered to have died from a definite or probable inherited cardiac disorder. Of the 157 relatives who underwent cardiac evaluation, 36 (23%) were diagnosed with a cardiac condition, which had not been previously identified.

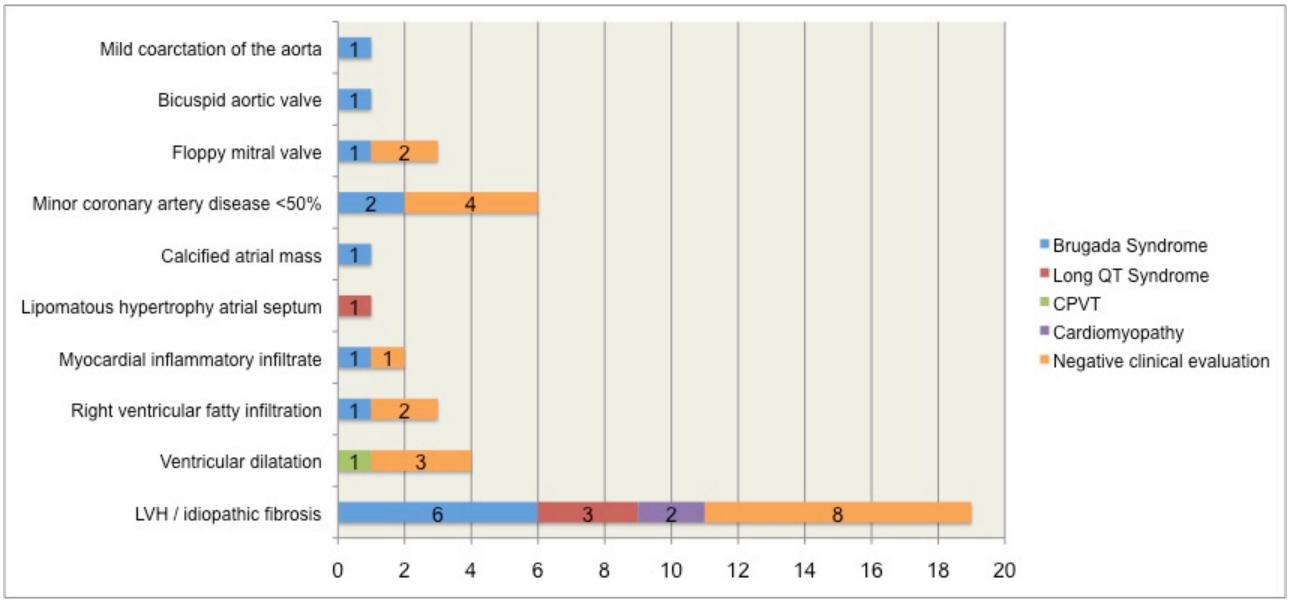


Figure 15: Histogram depicting the diagnostic yield of familial evaluation in victims of sudden cardiac death. The x-axis represents the number of families and the y-axis the pathology identified in the deceased during post mortem evaluation of the heart. The different colours within the columns represent the diagnosis established after cardiac evaluation of surviving relatives with absolute numbers of families stated within the relevant colour.

Abbreviations: CPVT: Cathecholaminergic polymorphic ventricular tachycardia; LVH, left ventricular hypertrophy.

5.5.3 Diagnosis of arrhythmogenic syndromes

A hereditary arrhythmogenic syndrome was diagnosed in the majority of families (19 of 21) in whom an underlying inherited cardiac condition was identified. Brugada syndrome (n=14) was the predominant diagnosis, followed by LQTS (n=4) and a single case of CPVT.

An arrhythmogenic syndrome was detected following familial evaluation in 42% (11/26) of cases where the autopsy findings were suggestive of a possible cardiomyopathy (LVH,

myocardial fibrosis, ventricular dilatation, or myocardial fatty infiltration) (Figure 15). In these cases, all relatives with an arrhythmogenic syndrome phenotype underwent CMR scans, in addition to standard evaluation, to exclude potential myocardial disease mimics. All CMR scans were reported as normal. Of interest, in the 19 SCD cases where LVH or myocardial fibrosis (isolated LVH: n=10; myocardial fibrosis alone: n=6 or in conjunction with LVH: n=3) was reported at post-mortem, evaluation of family relatives identified an arrhythmogenic syndrome in almost 50% of families (5 out of 10 cases with isolated LVH and 4 out of 9 cases with myocardial fibrosis) (Figure 15). A cardiomyopathy was diagnosed in only one case in either group. In the remaining 8 (42%) cases we were unable to identify any features of inherited cardiac pathology despite comprehensive evaluation of the deceased's relatives.

Brugada syndrome was also diagnosed in one of the three families whose proband exhibited isolated fatty infiltration of the right ventricle (Figure 16.4). One of the families where the pathologist reported marked right ventricular dilatation was subsequently diagnosed with CPVT, based on the identification of typical bi-directional ventricular tachycardia on exercise testing in two relatives.

Moreover, 2 out of the 6 families whose probands exhibited atheromatous disease at post-mortem were diagnosed with BrS. Both probands were young, aged 28 and 34 years-old respectively, and exhibited up to 50% coronary artery lesions in the left anterior descending and right coronary arteries (Figure 16.1). In one of the two families where an inflammatory infiltrate commonly attributed to myocarditis was present, BrS was diagnosed during Ajmaline provocation testing in the deceased's father. In similar fashion, one of the three families whose proband exhibited pathological features of mitral valve prolapse was

subsequently diagnosed with BrS based on the presence of type-1 Brugada ECG in two relatives.

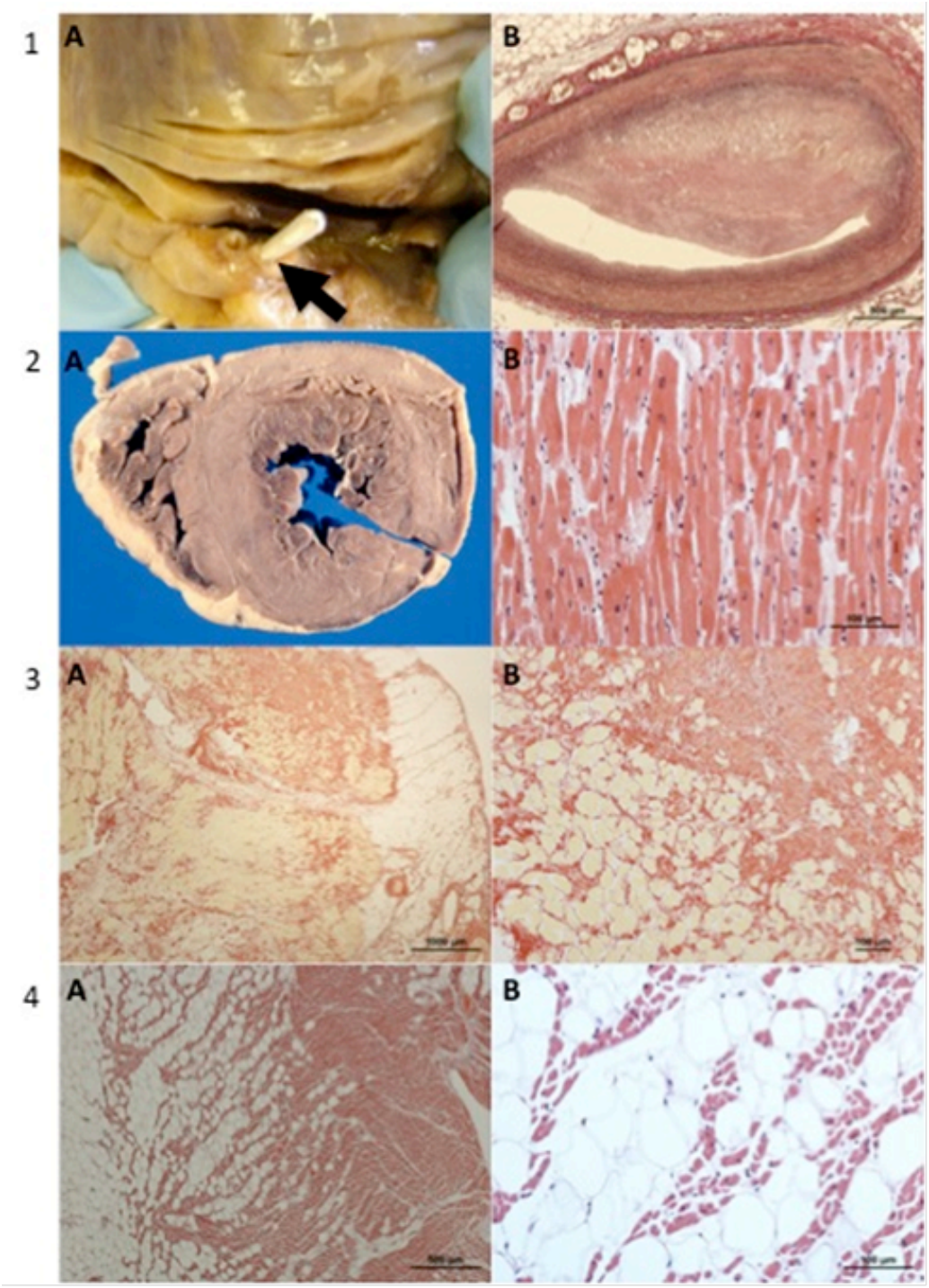


Figure 16: Histopathological slides of individuals with autopsy findings of uncertain significance. 1. Histopathological slides of an individual who exhibited coronary artery disease on autopsy and subsequent familial evaluation identified Brugada

syndrome: A. Macroscopic examination of the left anterior descending coronary artery in an otherwise normal heart shows eccentric atheroma. This can be opened with a 2mm probe (black arrow), indicating that there would have been normal blood flow during life; B. Histology staining with Trichrome stain (Elastin Van Gieson) confirmed eccentric atheroma in the left anterior descending coronary artery. The coronary lumen has a collapsed appearance, but would have been likely to have had normal ante-mortem blood flow. 2. Histopathological slides of an individual who exhibited isolated left ventricular hypertrophy on autopsy and subsequent familial evaluation identified Long-QT syndrome: A. Short axis slice showing a circumferentially thickened left ventricular wall measuring 2cm. The left ventricular cavity diameter is also reduced; B. Haematoxylin and eosin staining confirms idiopathic myocyte hypertrophy with enlarged box-shaped nuclei. No myocyte disarray is noted. 3. Histopathological slides of an individual who exhibited myocardial fibrosis on autopsy and subsequent familial evaluation identified Brugada syndrome: x2 (A) and x10 (B) magnification of picro-sirius red staining shows extensive myocardial replacement with collagen (stained red) in the left ventricular wall from epicardium into mid-myocardium. There is also fine interstitial collagen surrounding individual myocytes (yellow). Mild fatty infiltration is also noted within the collagen areas. 4. Histopathological slides of an individual who exhibited right ventricular fatty infiltration on autopsy and subsequent familial evaluation identified Brugada syndrome: x4 (A) and x20 (B) magnification of haematoxylin and eosin stain of the right ventricular wall showing significant fatty infiltration in the outer third of the myocardium (stained red). There is no fibrous tissue.

5.5.4 Diagnosis of cardiomyopathy

Only 2 families were diagnosed with an inherited cardiomyopathy based on the results of the familial evaluation; 1 dilated cardiomyopathy (DCM); and 1 HCM. The first case was of a 17-year-old boy who died in his sleep. The post-mortem revealed circumferential subendocardial haemorrhage with extensive myocardial fibrosis of the left ventricle. Evaluation of his relatives revealed a dilated, globally hypokinetic left ventricle in his mother and one of his sisters. The second case was of a 20-year-old male who died at rest. The post-mortem revealed a heavy heart (>500g) with LVH but no evidence of myocardial fibrosis or myocyte disarray. There was no documented history of hypertension or regular exercise. Familial evaluation revealed asymmetric septal hypertrophy in the context of a non-dilated left ventricular cavity in his father, raising suspicion of HCM. Unfortunately the father declined further investigations with CMR and genetic testing.

5.5.5 Mutation analysis

Mutation analysis in relatives with phenotypes suggestive of inherited cardiac conditions was undertaken in 17 out of the potential 21 families. In 2 families (1:HCM, 1:BrS), individuals declined genetic testing after counselling. In 2 LQTS families genetic testing was performed by their local geneticist. Due to the absence of co-existing atrio-ventricular block, mutation analysis was not performed in the family diagnosed with DCM.[214] Of the 13 families with BrS who underwent genetic testing, three carried pathogenic *SCN5A* mutations (*R376H, H558fs, A1680T*). Pathogenic mutations were also identified in the two LQTS families tested (E1784K and G840R in *SCN5A*) and in the CPVT family (A4556T in *RYR2*). Four of the identified mutations are previously reported as disease-associated (*SCN5A* R376H, A1680T, E1784K and RYR2 A4556T).[218,219] One novel *SCN5A* mutation

(H558fs) is a deletion resulting in a frame-shift, while the other (G840R) is a missense mutation with in-silico confirmation of disease-causation.[223,224] The post-mortem findings of the 6 families with a positive genotype are tabulated in table 17.

Table 17: Detailed presentation of the characteristics of victims of sudden cardiac death, including post-mortem findings, and relatives diagnosed with a condition in families where the presence of an inherited arrhythmogenic syndrome was confirmed by the presence of a pathogenic mutation in the relatives.

Victims age, gender, mode of death	Post-mortem findings	Clinical phenotype Pathogenic mutation identified in relatives	Clinical findings in relatives diagnosed with a hereditary arrhythmogenic syndrome
27 years Female At rest	Septal subendocardial fibrosis	LQTS SCN5A:p.G840R	QTc (Bazett) of 479ms^{½} (65bpm) and non-sustained VT in father, in context of family history (Schwartz score 4.5). Brother has QTc of 450ms^{½} (84bpm) with prolongation late in recovery post-exercise (Schwartz score 2).
34 years Male At rest	Lipomatous hypertrophy of atrial septum	LQTS SCN5A:p.E1784K	Resting QT prolongation in 2 daughters (Bazett QTc 497ms^{½} at 70bpm and 490ms^{½} at 71bpm) with syncope in one consistent with Schwartz score 4 in both.
17 years Male At rest	Marked right ventricular dilatation	CPVT RYR2:p.A4556T	Exertional polymorphic non-sustained ventricular tachycardia in mother; bidirectional ventricular couplets and triplets in sister.
39 years Male Cycling	Mitral valve prolapse (floppy mitral valve with mild ballooning)	BrS SCN5A:p.H558fs	Type-1 Br phenotype post Ajmaline in father and brother of deceased. Brother had experienced 2 episodes of unheralded syncope.
30 years Male At rest	Left ventricular Hypertrophy. Maximal wall thickness 18mm No significant disarray	BrS SCN5A:p.R376H	Type-1 Br phenotype post Ajmaline in father and paternal aunt of deceased. Father exhibited inducible Br phenotype and ventricular ectopy post exertion during exercise test.
37 years Male Asleep	Calcified atrial mass	BrS SCN5A:p.A1680T	Type-1 Brugada ECG post Ajmaline in sister of deceased.

5.5.6 Prevalence of early repolarization

Of the 157 relatives evaluated, 10.2% (n=16) exhibited the ER pattern in the inferior and/or lateral leads and less than 2% (n=3) demonstrated a slurred terminal QRS complex with a descending ST-segment, which is the ER pattern predominantly linked to the risk of sudden death in the general population.[186] Although the prevalence of ER was higher in relatives without a diagnosis of arrhythmogenic syndrome or cardiomyopathy compared to relatives with a diagnosis, the difference did not achieve statistical significance (11.6% versus 5.6%, p=0.366). Only one family consisted of ≥2 relatives who exhibited ER. In that particular family the deceased's post-mortem revealed mitral valve prolapse and no diagnosis was established after comprehensive cardiac evaluation of blood relatives.

5.5.7 Immediate management

All affected 36 relatives received appropriate life style modification and drug avoidance advice. Eleven patients were prescribed beta-blockers: 7 LQTS; 2 CPVT; 2 DCM. The two DCM patients were also initiated on an angiotensin converting enzyme inhibitor treatment. Prophylactic cardioverter defibrillators (ICD) were implanted in five patients: 3 BrS; 2 LQTS and two LQTS patients received a pacemaker.

5.6 Discussion

In a significant proportion of SCDs the pathologist may observe findings that are relatively common in the general population, or those that only partially fulfil diagnostic criteria for structural cardiac disease, leaving uncertainty with regard to causality and further management of the surviving relatives. In this study of 41 families of patients with SCD

with post-mortem findings of uncertain significance, 46% were diagnosed with a hereditary arrhythmogenic syndrome. This yield is similar to the diagnostic yield in our large, "true" SADS cohort, where primary arrhythmogenic syndromes accounted for 42% of deaths (p=0.703). (study IV) The causes of SCD were also similar, with BrS accounting for the majority of the diagnoses in both cohorts (34% in the cohort with post-mortem findings of uncertain significance versus 36% in the SADS cohort; p=1.00). Additionally, a similar proportion of the relatives evaluated in both the autopsy findings of uncertain significance and the SADS cohorts were diagnosed with a cardiac condition (23% versus 24%, p=0.906). This is of particular importance since by convention the absence of any cardiac pathology is regarded a prerequisite for the definition of a death as SADS.[7,31]

5.6.1 Implications of autopsy findings of uncertain significance

The causal effect of the autopsy findings is unclear. The authors offer four plausible hypotheses:

(a) Innocent bystander

Bicuspid aortic valve and floppy mitral valve are present in 1-2% of the general population and may represent innocent bystanders. Likewise, coronary atherosclerosis without significant narrowing of the arterial lumen and without macroscopic or microscopic evidence of acute or chronic ischaemia is common. It is well documented that the degree of coronary artery stenosis can be overestimated by the pathologists as a result of post-mortem collapse of the vessel wall. This was also evident in our study of comparing the results of post-mortem evaluation between general and expert cardiac pathologist (study III), where coronary artery atheroma was considered a significant cause of death in 3

cases by the referring pathologist but was determined to be non-significant by our expert cardiac pathologist who reassigned all 3 cases as SADS deaths. Support for this hypothesis is evidenced by the fact that out of the 6 victims who exhibited minor coronary artery disease, 5 died at rest. Paradoxically, in the remaining case that died post exertion, familial evaluation diagnosed BrS in 5 relatives (Figure 16.1). Finally, foci of lymphocytes are common in the normal heart and a degree of myocardial inflammation may be the effect of prolonged resuscitation efforts in young individuals rather than evidence of myocarditis resulting in SCD. This is again highlighted in study III, where 4 out of 9 cases assigned a diagnosis of myocarditis based on the presence of myocardial inflammation by the referring pathologist, were reassigned to a diagnosis of SADS by our expert pathologist.

(b) Primary cause of sudden cardiac death

Most of the conditions identified at autopsy in our cohort have been associated with malignant ventricular arrhythmias and sudden death. Sudden cardiac death in both athletic and non-athletic individuals have been attributed to mitral valve prolapse and myocarditis.[14] Similarly the absence of severe luminal narrowing of the coronary arteries does not preclude ventricular arrhythmias due to myocardial ischaemia,[273] particularly as a result of coronary artery vasospasm[274] and isolated fatty infiltration involving the cardiac conduction system has been implicated in sudden death of obese people.[275]

(c) Trigger in the context of an arrhythmogenic syndrome

Consideration must also be given to the fact that structural cardiac disorders may serve as triggers for malignant arrhythmias in the context of a coexistent inherited arrhythmogenic

syndrome. One-third of SCD cases in our cohort with minor coronary disease were subsequently attributed to an arrhythmogenic syndrome following familial evaluation. Current evidence suggests that the presence of coronary artery disease is an independent risk factor for LQTS-related symptomatic events.[276] It appears likely that transient ischaemia alters the arrhythmic substrate in susceptible individuals, by reducing the threshold for after-depolarisations or increasing transmural dispersion of repolarization, both recognized mechanisms for arrhythmogenesis in ion-channel disease.[277]

(d) Spectrum of arrhythmogenic syndromes

There is mounting evidence that individuals with ion-channel defects may exhibit structural cardiac changes.[277] The majority of BrS patients possess a structurally normal heart, consistent with the notion that this is a primary electrical heart disease. A minority, however, appears to exhibit evidence of ventricular wall motion abnormalities, ventricular dilatation, myocardial inflammation and fibrosis.[278,279] Such structural abnormalities may be subtle and echocardiography may appear normal, requiring more sophisticated diagnostic tools including CMR, positron emission tomography, and endomyocardial biopsy.[280,281] Several theories have been postulated to correlate ion-channel dysfunction with structural abnormalities, ranging from impaired excitation-contraction coupling and energy production to a hibernation-like state, which over time may even lead to intracellular lipid accumulation.[277] Support for potential structural abnormalities in patients with BrS despite normal conventional imaging is also lent by the study of Nademanee et al. where the authors identified the anterior aspect of the RVOT epicardium as the substrate for the Brugada type 1 ECG pattern.[282] Future correlation with detailed pathological evaluation of this area may provide further insights to the exact pathophysiology of BrS.

Additionally, there are reports in the literature of identical mutations presenting with either a cardiomyopathy or an arrhythmogenic syndrome phenotype, suggesting that structural and ion-channel defects may be part of a spectrum incorporating myocardial disease and primary arrhythmogenic syndromes. Mutations in the cardiac ryanodine receptor gene (RYR2), commonly implicated in CPVT, have been reported in individuals exhibiting an ARVC phenotype.[283] Moreover, a mutation in the SCN5A gene, implicated in BrS, may present with arrhythmia, conduction disease and atrial or ventricular dilatation.[284]

5.6.2 Left ventricular hypertrophy and myocardial fibrosis

In our cohort isolated LVH and myocardial fibrosis were by far the most prevalent findings. Idiopathic LVH is an increasingly recognized entity in cases of SCD and was the only finding in almost a third of post-mortems in our athletic cohort (study II). It remains unclear at this stage whether it represents an innocent bystander, a pathological variant of physiological LVH in genetically predisposed individuals or part of the spectrum of HCM. Although LVH is a well-recognized feature of cardiovascular adaptation to exercise,[57,72] in our study only 4 out of the 10 individuals exhibiting isolated LVH exercised on a regular basis. Data from the Framingham study also indicate that individuals with LVH have a four-fold risk of sudden death compared to controls without LVH.[285] In addition, experimental studies suggest that LVH alters ion-channel expression and function in a similar fashion to ion-channel disease, predisposing to re-entry arrhythmias and ventricular fibrillation.[286] Although in the majority of individuals such adaptations are unlikely to result in increased risk of arrhythmias, the development of LVH in an individual with an underlying hereditary arrhythmogenic syndrome may exacerbate electrical instability and predispose to sudden death.

Myocardial fibrosis also predisposes to malignant arrhythmias. Both the amount of fibrosis and the collagen texture appear to play a role in vulnerability to arrhythmia.[287] Moreover, myocardial fibrosis may represent incomplete expression of underlying cardiomyopathy, including the recently recognized arrhythmogenic left ventricular cardiomyopathy. Finally, myocardial fibrosis has been reported in marathon runners and in cases of SCD in athletic individuals raising concerns whether prolonged arduous exercise can lead to repeated myocardial injury, necrosis and subsequent fibrosis.

5.6.3 Early repolarization

The prevalence of ER pattern in the inferior and/or lateral leads in our cohort was lower than that observed by Nunn et al., and comparable to the prevalence reported in healthy controls.[188] More importantly, only 1 of the 41 families included more than one member with the ER pattern, offering little support to the theory of a potentially inheritable pro-arrhythmic trait.

5.6.4 The role of the cardiac pathologist

This study further highlights the importance of accurate interpretation of the autopsy findings by the pathologist since false conclusions may misguide familial evaluation to a probable diagnosis of structural disorders or offer false reassurance to surviving relatives and dissuade physicians from initiating familial screening altogether, with potentially devastating consequences in future generations. Given the relative rarity of SCDs from inherited conditions and the challenges associated with their diagnosis, the authors propose that all cases of SCDs, and particularly SCDs in young (≤35 years) individuals,

where an inherited condition is suspected or diagnostic uncertainty remains, as to the cause of death, should be referred for further evaluation to an expert cardiac pathologist.

5.6.5 Limitations of clinical evaluation of families

The limitations of the current study reflect the limitations of our investigative protocol and predominance of BrS and are very similar to the limitations reported in chapter 4. As with the "true" SADS cohort the predominant diagnosis in the cohort of individuals with autopsy findings of uncertain significance was of BrS. The authors concede that given the relative novelty of the condition, the considerable impact of the higher intercostal leads in the diagnostic yield and the association of the Brugada phenotype with structural cardiac abnormalities, it is possible that some of the relatives exhibiting the Brugada phenotype did not have a genuine arrhythmogenic syndrome. However, all individuals who were diagnosed with BrS underwent comprehensive evaluation including a detailed echocardiogram and a significant proportion were subjected to CMR, based on the histopathologic features identified in the deceased, and none exhibited any evidence of structural cardiac anomalies. Further support for the presence of BrS is underscored by the genetic yield (25%) of pathogenic SCN5A mutations, which is similar to existing literature.[106]

The omission of regular epinephrine testing in individuals without an established diagnosis despite comprehensive cardiac screening may have underestimated the prevalence of CPVT.[81] Finally, in the families in whom a pathogenic mutation was identified, we were unable to perform post-mortem analysis in the tissues of the victims for confirmation of the genotype since no tissue was available by the time the relatives were evaluated in our clinic.

Chapter 6: The impact of higher intercostal leads in the diagnosis of Brugada syndrome

6.1 Introduction

The majority of studies in patients with BrS have relied on the presence of the Type-1 ECG Brugada pattern in the conventional right praecordial leads with leads V1 and V2 placed on the 4th intercostal space. Shimizu et al.[225] performed an eighty-seven-lead body surface potential mapping with simultaneous 12-lead ECGs recordings in 25 patients with BrS and 40 control patients. The amplitude of the ST segment was measured from all 87 leads, and an ST isopotential map was constructed. The maximum ST elevation was distributed in an area of the right ventricular outflow tract in all Brugada patients, and it was larger than that in control patients (0.37 ± 0.13 versus 0.12 ± 0.04 mV; $p < 0.001$). In 18 (72%) of the Brugada patients who exhibited the typical type-1 Brugada pattern in leads V1 and V2 of the standard 12-lead ECG, the maximum ST elevation was observed on the level of the 4th intercostal space. In the remaining seven patients only a mild saddleback-type ST elevation was seen in leads V1 and V2 on the 4th intercostal space but the typical Brugada phenotype was observed in leads V1 and V2 recorded on the second or third intercostal space. In those patients the maximum ST elevation was located on the second intercostal space. The authors concluded that leads V1-V3 should be recorded on both the 4th and the higher (2nd and 3rd) intercostal spaces, when BrS is suspected.

A further study evaluated the usefulness of ECG recordings in the 3rd intercostal space.[288] The authors recorded ECGs in both the 4th and 3rd intercostal spaces in 17 patients with a diagnosis of BrS and 206 consecutive males, who acted as the control group. In the Brugada patients group the prevalence of the type-1 Brugada pattern increased from

23.5% to 64.7% when an ECG was recorded in the 3rd ICS. In the control group, the type-1 Brugada phenotype was present in 1 (0.5%) individual at baseline ECG and 2 (1%) individuals at the higher intercostal leads. In only 1 of the 206 controls did a normal baseline ECG convert to a diagnostic pattern by placing leads V1 and V2 in the higher intercostal spaces.

A subsequent study by Miyamoto et al.[226] evaluated the prognostic value of the type-1 Brugada phenotype recorded on the higher intercostal leads compared to the conventional 4th intercostal space. The authors evaluated 98 male individuals with a diagnosis of BrS. They divided them in 3 groups: 68 individuals who had a spontaneous type-1 Brugada pattern in the standard V1 and V2 leads; 19 individuals who exhibited the type-1 ECG in the higher intercostal leads only; and 11 individuals who exhibited the type-1 pattern only during provocation testing with a class 1 antiarrhythmic. There were no significant differences in baseline clinical characteristics. During prospective follow-up of about 2 years a similar proportion of cardiac events (documented VF, syncope or SADS) occurred in men with the Brugada pattern in the standard ECG leads and in men with the Brugada pattern in the higher intercostal leads (16% versus 11%; p=0.725). None of those who exhibited the Brugada pattern after provocation testing, only, suffered any cardiac events. Even in high-risk individuals with previous episodes of VF, subsequent cardiac events occurred in 44% of men with the Brugada pattern in the standard ECG leads and 50% of men with the Brugada pattern in the higher intercostal leads (p=1.00). In conclusion, men with a spontaneous type-1 Brugada pattern had similar prognosis, irrespective of whether the Brugada phenotype was identified in the conventional or the higher V1 and V2 leads.

A number of studies have also assessed the impact of higher intercostal leads during provocation testing with class 1 antiarrhythmics.[227,289,290] In the first study Sangwatanaroj

et al.[227] performed a Procainamide provocation test in 10 SADS survivors (individuals who survived a cardiac arrest as a result of documented VF or polymorphic VT and subsequent clinical evaluation failed to identify an underlying cause) and 48 of their relatives who had a normal baseline ECG. Electrocardiograms were recorded with leads V1 and V2 recorded in both the 4th and the higher (3rd and 2nd) intercostal spaces. Thirteen spouses served as the control group. After the Procainamide infusion, the type-1 Brugada phenotype could be demonstrated in 7 SADS survivors (70%) and 7 (14·6%) relatives with the standard ECG and in 9 (90%) (P = 0·26) and 23 (47·9%) (P = 0·0004) with the additional six-lead ECG, respectively. All the controls were negative for the Brugada sign. Based on these results it appears that raising the position of the V1 and V2 leads increases the sensitivity of the Procainamide challenge in detecting the Brugada phenotype, without compromising specificity. It should be emphasized, however, that the sample sizes were small, particularly the control group, and all positive Procainamide tests were presumed to be true positive results. None of the individuals was subjected to genetic testing to confirm a pathogenic mutation. A study by Meregalli et al.[289] reported on 160 Flecainide provocation tests performed in subjects considered to be at risk of BrS (n=82 as part of family screening). Of the 160 tests, 64 unmasked the diagnostic Brugada phenotype. All Flecainide tests were performed with the conventional ECG leads. The authors had the opportunity to perform ECGs utilizing the higher intercostal leads with leads V1 and V2 placed in the 3rd intercostal space, in 47 of the 64 positive tests and in all of the negative tests. In 21 of the 47 (45%) individuals with a positive test, a type-1 ECG was only obtained when the 3rd intercostal leads were used, while in 26 of the 47 (55%) a type-1 ECG was also recorded in the conventional leads. Of note, the proportion of mutation carriers was similar in both groups: 9/21 (43%) in the higher intercostal leads group and 10/26 (38%) in the conventional leads group ($p = 0.8$).

Finally, a recent study by Govindan et al.[290] evaluated the utility of higher intercostal leads during Ajmaline testing for the diagnosis of BrS. The authors performed 183 Ajmaline tests in individuals suspected of having the BrS. All ECGs were performed with leads V1-V3 placed at the conventional position. A repeat ECG was then performed with leads V1-V3 placed in the 3rd intercostal space. Towards the end of the study the authors used a 15-lead configuration, omitting lead V3 based on their own and others observation of its poor diagnostic yield,[211] and utilizing 6 V1 and V2 leads placed in the 4th, 3rd and 2nd intercostal space. Of the 183 tests, 31 (17%) were positive based on the second consensus criteria.[106] In all positive studies, at least one high lead became positive. In 13/31 (42%) cases, the type-1 ECG pattern could be observed only in the high right praecordial leads. Standard or high V3 were never positive before standard or high V1-V2. In seven patients, a type-1 pattern was seen in one standard and one high right praecordial lead (vertical relationship). The authors concluded that high V1-V2 leads are valuable for the diagnosis of BrS and appear to increase the detection rate by about 35%, without compromising specificity, either at baseline or during provocation testing. A vertical relationship of type-1 ECG patterns also appears to have a similar implication to a standard horizontal relationship. Use of the conventional 12-lead ECG alone may therefore result in loss of important diagnostic information.

6.2 Aim

There is a paucity of data relating to the impact of utilizing higher intercostal leads on the diagnostic yield of BrS during familial evaluation after a SADS death. The authors performed a study to compare the diagnostic yield of the Brugada phenotype utilizing the conventional V1 & V2 leads placed in the 4th intercostal space versus higher intercostal

leads (V1 & V2 placed in the 3rd and/or 2nd intercostal space) during comprehensive clinical evaluation.

6.3 Personal contribution

The author performed prospectively the clinical evaluation of all families, including performing or supervising, analyzing and databasing the majority of investigations (ECG, echocardiography, exercise treadmill test, Ajmaline provocation test and Holter monitoring). Collected data on the deceased including data on previous admissions or GP consultations. Analysed and reported all data.

6.4 Methods

Study cohort

The study cohort included 31 families, comprised of 133 relatives, who received a diagnosis of SADS secondary to BrS or Brugada overlap syndrome during the studies in chapter 4 ("true" SADS families) and chapter 5 (autopsy findings of uncertain significance), where relatives had undergone a baseline ECG and Ajmaline testing, as appropriate, with both the conventional and higher lead configuration. Only families assessed in Prof S Sharma's inherited cardiac diseases clinics were included in this study.

Familial evaluation

Family relatives were evaluated as per the methodology in chapter 4. As outlined in chapter 4, since the beginning of the thesis in October 2007 all individuals assessed in our cardiogenetics clinics were subjected to a baseline 12-lead ECG with leads V1 and V2

placed in the conventional 4th intercostal space as well as the 2nd intercostal space. Towards the end of the thesis ECGs were also performed with leads V1 and V2 placed in the 3rd intercostal space. Ajmaline provocation tests were also performed with the same ECG lead configuration. Ajmaline testing was considered the gold standard test for the diagnosis of BrS in the context of this study given the lack of alternative diagnostic tools and the limitations of genetic testing.[106] As such all Ajmaline provocation tests were considered to represent a true positive and true negative result, respectively.

Ethical approval

Data collection was performed as part of the clinical evaluation of the families as proposed by the Government in the 8th chapter of the National Service Framework for Heart Disease, which includes guidelines for early identification of individuals at risk of sudden cardiac death and better support for families of victims.

Statistical analysis

Data interpretation and analyses were performed using SPSS software, version 14 (SPSS Inc., Chicago, IL, USA). Means and standard deviations (SD) or median and interquartile range (IQR) were calculated for continuous variables. Group differences are examined using t-test and Mann–Whitney U test for parameters with normal and non-normal distributions, respectively. Chi-square or Fisher's exact test was used to test group differences of proportions. A value $p<0.05$ was considered statistically significant throughout.

6.5 Results

6.5.1 Baseline ECG

We compared baseline ECG features in leads V1 and V2 with leads placed in the conventional 4th intercostal and higher (3rd and/or 2nd) intercostal spaces (Table 18). Although raising the lead position did not affect the prevalence of ST-segment elevation (defined as ≥1mm of ST-elevation) to any significant degree, it increased considerably the prevalence of partial-RBBB and T-wave inversion. Of the 133 relatives, 56 exhibited the diagnostic type-1 Brugada phenotype. Only one individual had a highly suspicious baseline ECG with a type-1 Brugada pattern in lead V2.

Table 18: Comparison of baseline ECG phenotypes in leads V1 and V2 with leads placed in the 4th intercostal space (IS) and higher intercostal spaces (2nd &/or 3rd IS)

	4th IS n=133	Higher IS n=133	p-value
RBBB	2 (2%)	6 (4.5%)	0.282
partial-RBBB	14 (11%)	64 (48%)	<0.001
Left atrial enlargement	1 (1%)	21 (15.8%)	<0.001
T-wave inversion in V1	62 (47%)	105 (79%)	<0.001
T-wave inversion in V2	11 (8%)	33 (25%)	<0.001
ST-elevation in V1	10 (8%)	9 (7%)	1.000
ST-elevation in V2	24 (18%)	14 (11%)	0.114

Abbreviations: RBBB: Right bundle branch block

6.5.2 Ajmaline provocation test

One hundred and ten of the 133 relatives were subjected to an Ajmaline provocation test, based on the criteria reported in the methodology of chapter 4. Due to the lack of a control group from the general population, the authors used as controls relatives within the 31

families. Controls comprised of family members who were investigated as part of the family screening but were not genetically linked to the family members diagnosed with BrS. The controls were selected from families where both parents of the deceased were assessed and subjected to Ajmaline testing and comprised of 18 spouses of a parent of the deceased with a diagnostic Brugada phenotype and 2 half-siblings. The final groups for comparison comprised of 90 individuals in the study group and 20 individuals in the control group.

Of the 56 individuals diagnosed with BrS based on a positive Ajmaline provocation test, only 21 (38%) exhibited the Brugada phenotype in the conventional leads (4^{th} IS). On the contrary, the great majority of individuals (n=53, 95%) demonstrated the type-1 Brugada phenotype in the higher intercostal spaces (Table 19). Utilising the higher intercostal leads, increased the sensitivity of Ajmaline testing from 38% to 95%. On Ajmaline testing the presence of a type-2 and/or type-3 Brugada pattern was more common in the higher intercostal leads although it did not achieve statistical significance (17% in 4^{th} IS versus 26% in higher IS; p=0.141). None of the relatives assigned to the control group fulfilled the diagnostic criteria for a diagnosis of Brugada syndrome.

Table 19: Identification of the type-1 Brugada pattern at post-Ajmaline ECG with leads V1 and V2 placed in the 4^{th} intercostal space (IS) and higher intercostal spaces (2^{nd} &/or 3^{rd} IS)

Total No of Ajmaline tests (n=110)	Type-1 Br phenotype at 4^{th} IS	Type-1 Br phenotype at higher IS	p-value
Relatives (n=90)	21 (23%)	53 (59%)	<0.001
Control group (n=20)	0	0	1.000

On a family level, of the 31 families with a diagnosis of BrS or BrS overlap syndrome, 15 (48%) families had at least one relative who exhibited the diagnostic Brugada phenotype

in the 4th intercostal space. All 31 families however, had at least one relative with the Brugada phenotype in the higher intercostal leads ($p<0.001$).

There were no differences in the baseline characteristics of individuals (and families) exhibiting the type-1 Brugada phenotype in the higher intercostal leads alone, compared to those who exhibited the diagnostic phenotype in the conventional leads (Table 20).

Table 20: Characteristics of cohorts with a positive Ajmaline test with leads V1 and V2 placed in the 4th intercostal space (IS), compared to those with a positive test on higher intercostal leads (2nd &/or 3rd IS), alone.

Family level	Diagnostic at 4th IS n=15	Diagnostic at higher IS n=16	p-value
Age of death (mean)	29	29	0.883
Gender (Male)	87%	63%	0.220
Family history of SD (<50 years)	20%	25%	1.000
Genetic yield	3/8 (38%)	1/8 (13%)	0.570
Individual level	Diagnostic at 4th IS n=21	Diagnostic at higher IS n=35	p-value
Age (mean)	42	39	0.490
Gender (Male)	57%	51%	0.785
Symptoms - Syncope	33% 14%	34% 26%	1.000

Abbreviations: SD: Sudden death

In one family referred to our clinic after the death of a 23-year-old female at rest, both parents and the sister of the deceased were evaluated. The family was diagnosed with BrS based on a positive Ajmaline provocation test in the father. However the mother also exhibited the type-1 Brugada phenotype in a single lead, namely V2 placed in the 3rd intercostal space (Figure 17). Although the test was not considered diagnostic based on the absence of the type-1 Brugada pattern in ≥2 leads, the mother was advised to follow life-style modification advice as for BrS and further familial evaluation of both sides of the

family was recommended. Both parents were subjected to *SCN5A* genetic testing, which was negative in both cases.

Figure 17: ECG rhythm strips during Ajmaline provocation testing in the three family members of the deceased. The family received a diagnosis of BrS based on the presence of the type-1 Brugada phenotype in leads V2 in the 3rd and the 2nd IS. The case highlights the challenges of accurately interpreting the ECG findings. The mother of the deceased exhibited a type-1 phenotype in lead V2 in the 3rd IS. Moreover, both the mother and the sister of the deceased exhibited the type-3 phenotype in a number of leads.

Working on the assumption that all Ajmaline provocation tests were considered to represent true positive and true negative results and allowing for a single false positive test, the sensitivity of Ajmaline provocation testing improved from 38% to 95% by raising the position of leads V1 and V2, while the specificity reduced by a small degree only from 100% to 98%. Respectively, negative predictive value (NPV) increased from 61% to 95% while positive predictive value (PPV) reduced from 100% to 98%.

6.6 Discussion

The majority of studies in patients with BrS have relied on the presence of the Type-1 ECG Brugada pattern in the conventional right pre-cordial leads, with leads V1 and V2 placed on the 4th intercostal space. Studies by Shimizu et al.[225] and Hisamatsu et al.[288] were the first to demonstrate that a significant proportion of patients with BrS exhibit the diagnostic type-1 phenotype in the higher intercostal leads only, with leads V1 and V2 placed in the 3rd and/or 2nd intercostal space. Hisamatsu et al. demonstrated an impressive 3-fold increase of the prevalence of the type-1 Brugada pattern from 23.5% in the 4th ICS to 64.7% in the 3rd ICS. A number of studies have also assessed the impact of higher intercostal leads during provocation testing with class-1 antiarrhythmics and have demonstrated an increase of the diagnostic yield between 35% and 45%.[227,289,290]

To our knowledge this is the first study to report on the impact of higher intercostal leads in the context of systematic familial evaluation after a SADS death. In agreement with existing literature, we observed an increase of the diagnostic yield, based on the presence of the type-1 Brugada phenotype on Ajmaline provocation test, from 23% to 59%. While only 38% of individuals with a positive Ajmaline test exhibited the Brugada phenotype in the 4th IS, the great majority (95%) demonstrated the diagnostic pattern in the higher

intercostal leads. On a family level, of the families diagnosed with BrS 48% had at least one relative who exhibited the diagnostic Brugada phenotype in the 4th IS, while all families had at least one relative with the Brugada phenotype in the higher intercostal leads. Based on these observations the use of higher intercostal leads more than doubled our diagnostic yield of BrS. In absolute terms the diagnostic yield increased by almost 107% on a family level and 105% on an individual level.

6.6.1 Considerations relating to the use of the higher intercostal leads

Utilizing higher intercostal leads raises the potential for an increased false positive rate that artificially raises the diagnostic yield of BrS. Such concerns are reinforced by the higher prevalence of partial-RBBB and T-wave inversion in leads V1 and V2 by raising the lead position, as demonstrated in the 133 individuals who had baseline ECGs with both lead arrangements (Table 18). Given the relatively small number of individuals diagnosed with the condition, when one considers the poor genetic yield, we were unable to correlate pathogenic mutations with Ajmaline test results. As such, in the absence of a gold standard test for the diagnosis of BrS all positive Ajmaline tests were presumed to represent true positive results. Our results are however in keeping with existing literature which has consistently demonstrated that higher intercostal leads increase the sensitivity without significantly compromising the specificity of the ECG in detecting the Brugada phenotype, particularly in the context of provocation testing with a calss-1 anti-arrhythmic. In our cohort, raising the position of leads V1 and V2 increased the sensitivity of Ajmaline provocation testing by a factor of 2.5, from 38% to 95%. Only one individual exhibited a potentially false positive Ajmaline test in the higher leads (Figure 17) resulting in a very modest reduction of the specificity from 100% to 98%. This specificity is similar to the

specificity of 94.4% quoted by Hong et al.[178] for Ajmaline tests performed with the conventional leads in SCN5A mutation positive families.

The 2005 Heart Rhythm Society and European Heart Rhythm Association consensus document recommends that this group be treated no different than those with ECG changes in standard leads.[106] Evidence that the positive Ajmaline tests in the higher intercostal leads are likely to represent true positive results come from the study of Miyamoto et al.[226] which demonstrated that men with a spontaneous type-1 Brugada pattern had similar prognosis, irrespective of whether the Brugada phenotype was identified in the conventional or the higher V1 and V2 leads. In addition, Meregalli et al.[289] reported on 47 individuals with a positive Flecainide test, who underwent provocation testing at both the conventional and higher intercostal leads. The authors reported a similar proportion of mutation carriers in both groups (43% in the higher intercostal leads group versus 38% in the conventional leads group; p=0.80). In our study comparison between individuals with a positive Ajmaline test in the conventional versus the higher intercostal leads did not identify any significant differences either at family or at individual level. Our overall genetic yield was similar to existing literature.[106]

Chapter 7: Risk stratification in Brugada syndrome

7.1 Introduction

Patients with BrS who have survived a ventricular fibrillation cardiac arrest are recommended to receive an ICD for secondary prevention in light of the significant risk of recurrent events.[106,171] The other recognized high-risk group recommended for an ICD consists of patients with symptoms secondary to a presumed self-terminating malignant

arrhythmia such as unheralded syncope, nocturnal agonal respiration and seizures, as well as the presence of a spontaneous type-1 Brugada ECG pattern. [172,174,291] A number of studies have evaluated the additional value of specialist ECG and invasive assessments such as electrophysiological studies for the induction of ventricular arrhythmias in risk stratification, although conclusions regarding their impact have been inconsistent. [170-176,291]

The first study relating to risk stratification in BrS was published in 2002 by Brugada et al.[171] The authors reported on follow-up data of 334 patients with a diagnosis of BrS from an international registry. Based on the published manuscript a unified protocol was used during electrophysiological studies. The protocol used a single site of stimulation (right ventricular apex), three basic pacing cycle lengths (600, 500, and 430 ms), and induction of 1, 2, and 3 ventricular premature beats down to a minimum of 200 ms. A patient was considered inducible if sustained ventricular arrhythmias (ventricular fibrillation, polymorphic ventricular tachycardia, or monomorphic ventricular tachycardia lasting more than 30 seconds or requiring emergency intervention) were induced. The patients were grouped in individuals who were diagnosed after a cardiac arrest (group A; n=71), those who experienced episodes of syncope (group B; n=73) and asymptomatic individuals (group C; n=190). During 54±54, 26±36 and 27±29 months of mean follow-up, respectively, 62% of patients in group A, 19% of patients in group B and 8% of patients in group C had a new arrhythmic event defined as sudden death or documented ventricular fibrillation. Inducibility of ventricular arrhythmias during electrophysiological studies was an independent predictor of arrhythmia occurrence in all groups. Based on their results the authors quoted an incidence of malignant arrhythmic events of 13.7% per year in patients with aborted SCD and 8.8% per year in syncope patients. In asymptomatic individuals, a basal diagnostic ECG was a predictor of an arrhythmic event with 14% of individuals with a spontaneous type-1 Brugada pattern versus none of those who exhibited the Brugada

pattern only after provocation with a sodium channel blocker challenge, experiencing arrhythmia during follow-up. Of the asymptomatic individuals with a spontaneous Brugada pattern, a positive electrophysiological study was predictive of an arrhythmic event during follow-up.

In a further study based on the same registry led by Brugada et al. a total of 547 patients with BrS but no previous episode of aborted SCD were followed-up for a mean of 2 years.[174] Forty-five patients (8.2%) suffered sudden death or documented ventricular fibrillation. Multivariate analysis identified the inducibility of a sustained ventricular arrhythmia during electrophysiological studies as the strongest predictor of future malignant events (RR 5.9, 95%CI 2.0-16.7; p<0.0001), followed by a history of syncope (RR 2.5, 95%CI 1.2-5.3; p=0.017). During logistic regression analysis inducibility during electrophysiological studies conferred a significant additional risk even to asymptomatic individuals with a normal baseline ECG, with 0.5% of non-inducible but 4.5% of inducible patients exhibiting an event during the mean follow-up of 2 years. Although the authors conceded that a longer follow-up study would be necessary to draw safe conclusions, the current study indicated that most patients with an established diagnosis of BrS who are inducible during electrophysiological studies, are at sufficient risk to justify the implantation of an ICD.

In contrast to the registries led by Brugada et al.,[171,174] two subsequent reports from national and international registries failed to demonstrate a correlation between a positive programmed ventricular stimulation test and an increased risk of arrhythmic events. A review of an Italian registry of 200 BrS patients, 86 individuals underwent programmed ventricular stimulation, which failed to detect increased risk of arrhythmic events.[172] In this particular study the most reliable predictors of increased risk of sudden cardiac death were

the combination of a spontaneous ECG pattern and syncope (Hazard ratio of 6.4). The presence of a spontaneous ECG pattern on its own conferred an intermediate risk (Hazard ratio of 2.1), while the presence of syncope alone was not an independent predictor of risk.

In 2010, the results of the FINGER registry were published.[176] The registry included 1029 consecutive individuals with a diagnosis of BrS, including previously published cohorts.[292,293] An electrophysiological study was performed in 638 patients and included maximum of 3 ventricular extrastimuli delivered to 2 ventricular sites. During a median follow-up of 32 (14 to 54.4) months, 5% (n=51) of patients experienced cardiac events (44 patients experienced appropriate implantable cardioverter defibrillator shocks and 7 died suddenly). The cardiac event rate per year was 7.7% in patients with aborted SCD, 1.9% in patients with syncope, and 0.5% in asymptomatic patients. In agreement with the Italian registry data, a spontaneous type-1 ECG conferred increased risk of arrhythmic events, whereas gender, family history of SCD, inducibility of ventricular tachyarrhythmias during electrophysiological study, and the presence of an *SCN5A* mutation were not predictive of arrhythmic events. In contrast to the Italian registry however, the FINGER study also identified the presence of syncope as an independent risk factor, with or without the presence of a spontaneous type-1 pattern.

As all registry reports are based on multicentre data, criticism has been raised relating to the potential use of different stimulation protocols. However, results from single centre studies have also been conflicting even when similar stimulation protocols were utilised. Kanda et al.[170] studied 34 symptomatic patients with a diagnosis of BrS based on the presence of a spontaneous type-1 ECG pattern. All patients underwent programmed ventricular stimulation at 2-ms and twice the diastolic threshold current from the right ventricular apex and the RVOT, using two basic cycle lengths (500 and 600 ms) and a

maximum of three premature beats. Patients were classified into two groups according to the inducibility of VF: 22 patients with induced VF requiring direct cardioversion for termination (Induced VF group) and 12 patients without induced VF (Non-induced VF group). The great majority of patients had an ICD in situ allowing for accurate documentation of arrhythmic events. During a mean follow-up of 38 months (range 1 to 149 months) there was no significant difference in the recurrence of cardiac events (documented VF or sudden cardiac death) between the two groups (36% of induced VF versus 58% of non-induced VF; p=0.620).

Finally, the PRELUDE (programmed electrical stimulation predictive value) registry, led by Priori et al.[291] is the only prospective registry designed specifically to assess the value of programmed electrical stimulation and induction of ventricular tachycardia or fibrillation as a predictor of arrhythmic events in BrS. In contrast to some of the retrospective registries all the participants used the same stimulation protocol. During a median follow-up of 34 months, 14 arrhythmic events (4.5%) occurred. Programmed electrical stimulation induced ventricular tachyarrhythmias in 40% of patients but was not a predictor of events at follow-up. Of interest, of the 111 inducible patients who were subjected to a second electrophysiological study, in only 34% were the investigators able to reproduce the initial result. In agreement with previous registries, both the history of syncope and the presence of a spontaneous type-1 ECG were independent predictors of malignant arrhythmia, with the combination of the two being the strongest predictor (hazard ratio [HR]: 4.20). In addition, ventricular refractory period <200 ms (HR: 3.91) and QRS fragmentation (HR: 4.94) were significant predictors of arrhythmias.

Based on the interpretation of the available evidence, most specialist centres in the UK recommend the implantation of an ICD in patients with a diagnosis of BrS who have either

survived a cardiac arrest or have experienced symptoms secondary to documented or presumed self-terminating malignant arrhythmia. The great majority of asymptomatic patients are reassured of a relatively low risk of sudden death compared to the risk of potential complications from the ICD implantation and are treated conservatively with regular follow-up and the advice to report any new symptoms. Electrophysiological studies are rarely performed for risk stratification purposes, outside the context of research. Occasionally a negative study is used in very anxious asymptomatic patients in order to offer further reassurance relating to their low risk of sudden death.

7.2 Aim

Based on our experience, we were concerned that in the majority of SADS families where a diagnosis of BrS was assigned following familial evaluation, the SADS victims were not known to have high-risk features. As such even if the victims had been evaluated prior to their death they would not have been offered an ICD and the death would not have been prevented. We therefore systematically reviewed the data available on individuals who experienced SADS due to presumed BrS, established following familial evaluation, to determine the prevalence of conventional high-risk characteristics. The objective of this study was to determine the prevalence of conventional risk factors for sudden death, such as VF arrest, symptoms secondary to a presumed self-terminating malignant arrhythmia and the presence of a spontaneous type-1 Brugada ECG, in sudden arrhythmic death syndrome (SADS) probands with a familial diagnosis of BrS.

7.3 Personal contribution

The author performed prospectively the clinical evaluation of the 30 families assessed at Lewisham hosptial, including performing or supervising, analyzing and databasing the majority of investigations (ECG, echocardiography, exercise treadmill test, Ajmaline provocation test and Holter monitoring). Collected data on the deceased including data on previous admissions or GP consultations. The author reviewed all the data (form Lewisham and St George's hospitals) and assisted with data analysis and drafting of the published manuscript.

7.4 Methods

Study cohort

The study was performed in collaboration with an inherited cardiac disease clinic at St George's Hospital. Between 2003 and 2010, 49 consecutive families (Lewisham hospital, n=30; St George's hospital n=19) were diagnosed with BrS in the context of a SADS death. Families included in this study are families with a diagnosis of BrS from the studies reported in chapter 4 and chapter 5. The diagnosis was based on the identification of the type-1 Brugada pehenotype in at least 1 family member as previously described. A total of 50 probands were included, with 1 family having 2 individuals with confirmed SADS. In 2 families reviewed, the proband was older than 45 years. One of these 2 families had stereotypical Brugada ECG changes in more than 1 blood relative, thereby fulfilling the consensus statement diagnostic criteria.[106]

Familial evaluation and characteristics of probands

Criteria for a familial diagnosis of BrS and a detailed account of the clinical evaluation of relatives have been reported in chapters 4 and 5. The presence of symptomatic events in each proband were determined by interviews with all evaluated family members and review of medical examiner and coroner reports. Structured clinical questions regarding the presence of prior transient loss of consciousness, seizures, or faints were retrospectively coded as probable syncopal events for the study analysis. All decisions regarding relevance of symptoms described were made by 2 investigators (Dr Hariharan Raju and Dr Michael Papadakis), with disputed results adjudicated by a senior investigator. The presence of an antemortem ECG for all probands was sought by detailed questioning of evaluated family members. This included review of history of attendance at health screening events, any hospital attendance, or presence of any prior cardiovascular symptoms (palpitations or chest pain) that may have prompted an ECG. When any family members suggested the SADS proband may have attended for medical assessment prior to his or her death, the existence of an antemortem ECG was questioned by written communication to any medical professional involved in the proband's investigation. As with familial ECGs, all ECGs of probands taken before death were reviewed by 2 investigators for evidence of a spontaneous Brugada pattern.

Ethical approval

Data collection was performed as part of the clinical evaluation of the families as proposed by the Government in the 8th chapter of the National Service Framework for Heart Disease, which includes guidelines for early identification of individuals at risk of sudden cardiac death and better support for families of victims.

Statistical analysis

Data interpretation and analyses were performed using SPSS software, version 14 (SPSS Inc., Chicago, IL, USA). Means and standard deviations (SD) were calculated for continuous variables. Group differences are examined using t-test. Chi-square or Fisher's exact test was used to test group differences of proportions. A value $p<0.05$ was considered statistically significant throughout.

7.5 Results

Demographics

In total, 202 blood relatives of probands were cardiologically evaluated and contributed to the reported proband histories. Details of associated familial evaluation are provided in Table 21.

Table 21: Breakdown of SADS Familial Evaluation	
Number of family members evaluated	202
Mean no. evaluated per family ± SD	4.0±2.4
Total diagnosed with BrS	83
Mean no. Diagnosed with BrS per family ± SD	1.7±1.1

Demographic characteristics and reported symptoms in the included probands are summarized in table 22. The mean age of death of probands was 29.1±10.6 years (range 4 to 56 years). A predominance of male BrS deaths was noted (41 male; 82%). Circumstances of death were obtained for 46 probands. Of these, 18 deaths (39%) occurred during sleep, with a further 19 (41%) at rest during the daytime; only 5 (11%) occurred during or immediately after significant exertion.

Table 22: clinical characteristics of Sudden arrhythmic death syndrome probands with familial diagnosis of Brugada syndrome			
Clinical Presentation	**Syncope**	**Asymptomatic**	**Unknown**
No of probands	9	36	5
Male/Female	5/4	31/5	5/0
Age, yrs	29±16	29±10	31±6
Type-1 BrS pattern/no. of ECGs available	0/2	1/3	0/0
Family history of prior SCD	1 (11)	6 (17)	0 (0)
Died in sleep or rest	8 (89)	28 (78)	1 (20)
Definite mutation/SCN5A analysis	1/5	4/22	1/1

Values are n, mean ± SD, or n (%).

Genetic testing

Details of families for whom SCN5A mutation analysis was undertaken (n=28) are given in table 17. Of the 5 families with unequivocal mutations, 2 have mutations that have previously been reported as disease causing (E1784K) and 3 have highly probable novel mutations (I1377V, D349H and H558fs). Overall, unequivocal mutations have been found in 18% of families for whom SCN5A mutation analysis was undertaken.

Risk profile of probands

Antemortem ECGs were available for 5 probands (Figure 18), 1 of whom demonstrated a spontaneous type 1 pattern (Figure 18A) and was taken during presentation with gastrointestinal symptoms in a previously asymptomatic individual. A further proband had evidence of a prior resting type 3 Brugada pattern in just 1 right ventricular lead (Figure 18B). Both of these ECGs were taken more than 1 year before each proband's terminal event. None of the probands had undergone prior provocation testing for investigation of inducible Brugada ECG pattern or invasive electrophysiological assessment; none had a pre-established personal or familial diagnosis of BrS or other inherited cardiac disease.

Figure 18: Antemortem ECGs in 5 SADS probands with BrS.

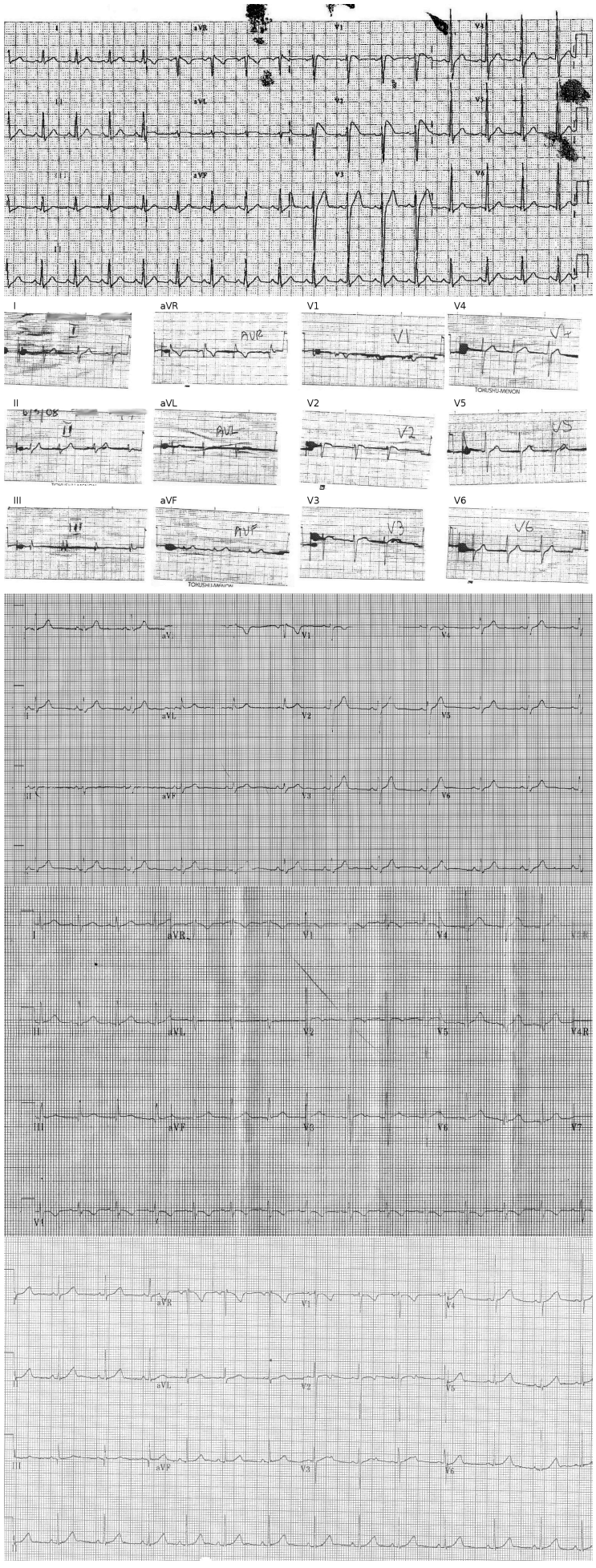
(a) The sole spontaneous type-1 antemortem ECG seen in our cohort of SADS probands with BrS.

(b) An antemortem borderline type-3 Brugada phenotype seen in lead V_2 only.

(c) A non-diagnostic antemortem adult ECG.

(d) An antemortem ECG taken at age 8 years, with no spontaneous Brugada phenotype.

(e) An antemortem ECG taken at age 4 years, with no spontaneous Brugada phenotype.



Probands' symptoms before death were reported reliably by family members in 45 cases, with the remainder uncertain of any prior medical history or symptoms. Only 9 of these 45 probands (20%) were reported to have experienced at least 1 syncopal episode before the fatal event. Seven probands (14%) had a prior family history of premature SCD, 1 of whom also had a personal history of syncope. Fifteen probands (30%) had either a prior family history of SCD or personal reported history of syncope. Among those who were previously symptomatic, 5 probands were male, whereas 4 were female.

7.6 Discussion

Current data regarding risk stratification in patients with BrS have predominantly been determined on the basis of short- and medium-term prospective cohort observation of those identified in life. The FINGER study remains the largest cohort studied thus far, with 1,029 consecutive patients and indicates that a prior cardiac arrest, spontaneous type-1 ECG, and syncope were the only independent indicators of arrhythmic risk in patients with the Brugada ECG.[176] This is a valuable tool in terms of primary prevention in a cohort setting. It is however less useful during consultation with individual families as the majority of sudden deaths in BrS are likely to occur in individuals at low risk, who comprise the overwhelming majority of individuals with BrS. In our cohort of SADS probands with BrS, only 18% (9 of 50) had a confirmed prior syncopal event, as determined by reported symptoms and medical history from relatives. This suggests that the majority of sudden deaths in BrS occurred in asymptomatic individuals. The absence of symptoms, however, does not necessarily ensure absence of significant prior arrhythmia. An observational study of ICD interrogations in 19 patients with BrS and prior aborted sudden death detected 64 episodes of ventricular fibrillation, 26 of which were asymptomatic by virtue of them being nocturnal and self-limiting, requiring no device discharge.[106] This evidently

limits the sensitivity of reported symptoms as a marker of prior ventricular arrhythmias. Furthermore, the specificity of syncope for ventricular arrhythmias among patients with BrS may be limited by the observation that there is a preponderance of other aetiologies of syncope, including increased susceptibility to significant vasovagal responses with head-up tilt testing.[294]

Although ECGs were available for a minority of probands, only 1 demonstrated a spontaneous type-1 pattern, calling into question the utility of its absence as a marker of low risk. The limited value of the ECG as a risk marker for SADS is further underlined by the knowledge that in the majority of Brugada gene carriers it may be normal or fluctuate between normal and the Brugada pattern.[158,168,169] This has important implications in the context of tertiary referral centres where individuals are likely to be evaluated once or at best on a small number of occasions.

Of the total cohort, only 18% were identified as fulfilling 2005 consensus[106] criteria for ICD implantation on the basis of prior syncope. A further 14% may have warranted risk stratification with electrophysiological study according to the consensus criteria because of the presence of a type-1 ECG before death (1 of 50) or a family history of prior SCD (6 of 50). Hence, current markers of risk for cardiac events and sudden death would have been insensitive, with at least 68% of our cohort categorized as low risk. Therefore, these markers may not have predicted the BrS deaths, even if a diagnosis of BrS had already been established. Current data suggest that these asymptomatic individuals' risk would have been low, $<$1% per year,[176] even if a spontaneous type-1 ECG pattern was seen. Given that current treatment is limited to ICD implantation, with its inherent complications in young patients, risk stratification in asymptomatic patients clearly requires improvement.

Limitations

Despite being the largest cohort of its kind reported, this study remains limited by the relatively small number of SADS probands with BrS included. Only 5 probands had a documented prior ECG in this study. Hence, it is difficult to make judgments on the presence or absence of a spontaneous type 1 Brugada ECG in the absence of prior investigation. This is an important consideration, given its apparent importance in risk stratification,[106] although syncope is a much more significant risk factor.[176] Unsurprisingly, in light of their predominantly asymptomatic status, none of the SADS probands had undergone comprehensive cardiological evaluation before the terminal event. It is also possible that probands may not have relayed any prior symptoms to family members and medical practitioners.

CONCLUSIONS OF THE THESIS

The thesis uses an integrative approach, involving clinical and laboratory disciplines, in an effort to improve our understanding of SADS and avert further deaths. The incorporation of data from the office of national statistics, the evaluation of cardiac tissue by a cardiac pathologist, the systematic clinical evaluation of family relatives in a specialist setting and the input of a clinical geneticist provide a comprehensive investigation of SADS.

The studies of the Office of National Statistics data (chapter 1) and the post-mortem evaluation of 118 athletic individuals (chapter 2) underscore the impact of SADS, which contributes a significant number of deaths per annum in the UK.

Evaluation of one of the largest cohorts of SADS families reported in the literature, confirms that a significant proportion of SADS deaths are caused by an underlying primary arrhythmogenic syndrome, highlighting the importance of recognition of such deaths and screening of all first-degree family relatives, to identify those at risk and avoid further tragedies. Given the novelty of some of the conditions implicated in SADS and their inherited nature we would recommend that familial evaluation be performed in specialist centres that adopt our “one-stop-shop” model. Our thesis also underscores the need for increased awareness among primary care physicians and specialists alike, to ensure recognition of cardiac conditions in young people since warning symptoms often precede SCD. Physicians should be vigilant since malignant arrhythmias might masquerade as epileptic seizures, atypical vacant episodes, near-drowning and unexplained road traffic accidents.

In contrast to published cohorts, this study identified BrS, a fairly novel primary arrhythmogenic syndrome, as the predominant cause of SADS. Our study highlights the value of higher intercostal V1 and V2 leads in the diagnosis of BrS, predominantly by increasing the sensitivity of the Ajmaline provocation test in detecting silent carriers. Based on our results we would recommend that all relatives of SADS victims, where no cardiac pathology is identified should undergo a baseline ECG and Ajmaline provocation testing with leads V1 and V2 placed in both the conventional and higher intercostal spaces.

Moreover, this thesis highlights certain knowledge gaps in our understanding of BrS, particularly in respect to risk stratification for sudden death and the potential detrimental effect of exercise. Our findings suggest that the great majority of SADS victims due to presumed BrS do not fulfill current criteria for consideration for an ICD. This places evaluating physicians in a difficult position, given the limited therapeutic options, and calls for an open-minded approach when advising patients relating their risk of sudden death. As more therapeutic strategies become available the use of ICD implantations will be limited to the patients at highest risk. In almost 10% of individuals affected by BrS, exercise can provoke the Brugada ECG phenotype and even result to ventricular extrasystoles during the recovery period, suggesting that is some cases exercise could be a potential trigger of malignant arrhythmias.

The comprehensive familial evaluation of victims of SCD with autopsy findings of uncertain significance has led the authors to re-evaluate the definition of SADS. Our results suggest that any death with autopsy findings that do not meet conventional criteria for structural heart disease should be considered a SADS death and first-degree relatives should undergo comprehensive evaluation including screening for primary arrhythmogenic

syndromes. This is of particular importance since by convention the absence of any cardiac pathology has been regarded a prerequisite for the definition of a death as SADS.

Finally, our collaboration with a specialist cardiac pathologist has highlighted the importance of an expert cardiac pathology service for the evaluation of victims of SCD and particularly SCD in young (≤35 years) individuals, where an inherited condition is suspected. Accurate interpretation of the autopsy findings is of utmost importance since false conclusions may misguide familial evaluation or offer false reassurance to surviving relatives and dissuade physicians from initiating familial screening altogether, with potentially devastating consequences in future generations. A prime example of the complexity associated with the interpretation of post-mortem findings and the potential implications is the entity of idiopathic LVH, which is increasingly recognised. Based on the results of our studies idiopathic LVH can be misinterpreted as HCM but may be associated with a range of conditions, including primary arrhythmogenic syndromes.

FUTURE WORK STIMULATED BY THE THESIS

The thesis has contributed to the existing scientific knowledge in the field of SADS but at the same time it has highlighted important scientific gaps that should further be explored in future projects. Our results are based primarily on clinical evaluation of family relatives with very limited contribution from genetic studies and in particular molecular autopsy. Existing studies have demonstrated that molecular autopsy can contribute to the diagnostic yield, although poor quality DNA samples, time-consuming techniques, financial burden and poor genetic yield hinder its widespread application. As such the field of molecular autopsy, particularly utilising fairly novel techniques such as next generation sequencing, remains largely unexplored.

It is clear from this study that our knowledge of risk stratification in BrS is limited and further studies that will explore novel risk markers are essential. Most studies seem to conclude that induction of VT or VF during electrophysiological testing is not a predictor of risk for SADS. Although novel ECG indices such as the width of the S-wave in V1, the amplitude of the ST-segment in V2 and fragmentation of the QRS complex have been suggested, these remain to be tested in large, prospective cohorts.[291,295-297]

Another potential area of interest which requires further exploration is the utility of Ajmaline provocation testing and the higher intercostal leads in diagnosing BrS. Given the impact of the higher intercostal leads and the potential implications of labelling an individual with a diagnosis of BrS it is imperative that we assess within the context of a large cohort: 1. The sensitivity and specificity of Ajmaline provocation test in the higher intercostal leads within a genotype positive population; 2. The sensitivity and specificity of higher intercostal leads (at baseline and during provocation testing) within a control cohort from the general

population; 3. Assess the influence of certain demographic factors such as gender; and 4. Assess the test-retest reproducibility of Ajmaline provocation testing.

Our study also confirmed the existence of idiopathic LVH, which was identified in a significant proportion of athletic individuals and individuals with autopsy findings of uncertain significance. We also demonstrated that idiopathic LVH may be associated with a familial diagnosis of HCM and primary arrhythmogenic syndromes and formulated a number of potential hypotheses as to the contribution of the hypertrophy in the sudden death. However, we did not have the opportunity to perform molecular autopsy in the victims and our numbers were fairly limited. A large study is required to clarify the exact significance of idiopathic LVH. Such a study should prospectively perform detailed histopathological evaluation of SCDs with idiopathic LVH, associated molecular autopsy and comprehensive clinical and genetic familial evaluation.

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Appendix 1: Abbreviations

ACE-i:	angiotensin converting enzyme inhibitors
ARVC:	arrhythmogenic right ventricular cardiomyopathy
AV:	aortic valve
BrS:	Brugada syndrome
BSA:	body surface area
CI:	confidence interval
CM:	cardiomyopathy
CMR:	cardiac magnetic resonance imaging
CPVT:	catecholaminergic polymorphic ventricular tachycardia
CRY:	Cardiac Risk in the Young
CTCA:	computed tomography coronary angiography
DCM:	dilated cardiomyopathy
DNA:	deoxyribonucleic acid
ECG:	electrocardiogram
HCM:	hypertrophic cardiomyopathy
ICD:	implantable cardioverter defibrillator
ICD-9:	International Classification of Diseases-ninth edition
ICD-10:	International Classification of Diseases-tenth edition
LBBB:	left bundle branch block
LQTS:	long-QT syndrome
LVH:	left ventricular hypertrophy
LVNC:	left ventricular non-compaction
LVWT:	left ventricular wall thickness
NCEPOD:	National Confidential Enquiry into Patient Outcome and Death

ONS:	Office of National Statistics
pRBBB:	partial right bundle branch block
PCR:	polymerase chain reaction
PolyPhen:	polymorphism phenotyping
QTc:	corrected QT-interval
RBBB:	right bundle branch block
SADS:	sudden arrhythmic death syndrome
SCD:	sudden cardiac death
SD:	standard deviation
SIFT:	sorting intolerant from tolerant
SQTS:	short-QT syndrome
UK:	United Kingdom
US/USA:	United States of America
VF:	ventricular fibrillation
VT:	ventricular tachycardia
WPW:	Wolff-Parkinson-White syndrome

Appendix 2: Definitions

Sudden death: Unexpected death (within 1–12 hours of apparent well being) from natural causes, of an apparently healthy individual, with no prior cardiac disease.

Sudden cardiac death: Sudden death where no extra-cardiac pathology is identified at post-mortem examination and a cardiac disease is identified or presumed as the most probable cause of death.

Sudden arrhythmic death syndrome: Sudden death where no definitive cardiac or extra-cardiac cause is identified despite detailed histopathological examination and toxicology screen. The death is presumed to be secondary to a malignant cardiac arrhythmia.

Athletic individual: An individual who participates in at least 2 hours per week of organised physical training and competes in regular team or individual sport.

Appendix 3: List of publications arising from the thesis

Europace (2009) **11**, 1353–1358
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CLINICAL RESEARCH
Sudden Cardiac Death Syndrome

The magnitude of sudden cardiac death in the young: a death certificate-based review in England and Wales

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Aims In the UK, the true impact of cardiac and sudden death in the young (≤35 years) is speculative. The authors critically appraised the office of national statistics (ONS) data for causes of death in the 1–34 years age group in England and Wales in an attempt to present an estimate of the incidence of such deaths and their underlying causes.

Methods and results The investigators analysed the ONS mortality data for 2002–2005, inclusive. International classification of diseases-10 codes representing possible cardiac deaths were selected and divided into four classes; A1: definite cardiac deaths with no structural heart disease identified at post-mortem, A2: definite cardiac deaths with structural heart disease identified at post-mortem, A3: definite cardiac deaths with indeterminate cause, and B: possible cardiac deaths. Analysis of the data revealed an average of 419 (SD 16.5) definite cardiac deaths per annum (Class A1 + A2 + A3) equating to 1.8 per 100 000 per year (SD 0.08) or 8 deaths/week. There were also 433 (SD 6.2) deaths per year in class B which comprised primarily of deaths from drowning and epileptic seizures. The most prevalent causes were ischaemic heart disease (33.5%), cardiomyopathies (27%), sudden arrhythmic death syndrome (14%), myocarditis (11%), valvular heart disease (5%), and hypertensive cardiomyopathy (2%).

Conclusion Our findings suggest that the number of cardiac and sudden deaths in the young identified is sufficiently high to command attention even without the inclusion of potential misclassifications (Class B). Awareness of such deaths among primary-care physicians, pathologists, and coroners should be raised to ensure that those at risk are identified and further tragedies are avoided.

Keywords Sudden cardiac death • Sudden arrhythmic death syndrome • Death certificates • Epidemiology

Introduction

Sudden cardiac death accounts for 50% of cardiovascular mortality with an estimated annual toll of 300 000 deaths in the USA and 60 000 deaths in the UK.[1,2] The majority of sudden cardiac deaths are of ischaemic aetiology secondary to atherosclerotic coronary artery disease and affect the older section (>35 years) of the population.[3] In a significant proportion of sudden deaths, no specific cause is identified despite detailed histopathological examination and toxicology screen, and a diagnosis of sudden arrhythmic death syndrome (SADS) is advocated.[3,4] The recognition of such deaths is of utmost importance since evaluation of first-degree blood relatives of the deceased commonly results in the identification of an ion-channel disorder and less frequently a cardiomyopathy in just over half of families evaluated, thereby providing a potential cause of death and identifying surviving relatives at risk.[5,6]

The epidemiology of sudden cardiac death in the young (≤35 years) is less well established. A population-based study in Minnesota reported an incidence of 6.2 per 100 000 per year among residents aged 20–40, whereas an autopsy review of US military recruits aged 18–35 revealed a rate of 13 per 100 000

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recruit-years.[7,8] Studies in the young athletic population in Italy and the USA have suggested much lower rates of sudden cardiac death with an incidence of 2.1 per 100 000 per year and 0.5 per 100 000 per year, respectively.[9,10] Inherited cardiomyopathies are the commonest cause of sudden cardiac death in athletes, whereas coronary artery pathology including coronary artery anomalies and atherosclerotic disease predominate in the non-athletic population.[7–9,11] The reported frequency of structurally normal hearts (SADS) varies widely, ranging from 3% in American young athletes to 35% in US military recruits.[8–12]

In the absence of a systematic national registry documenting sudden cardiac deaths in the young, the true impact of such fatalities in the UK is speculative. The objective of this study was to examine and critically appraise the office of national statistics (ONS) data for causes of death in the 1–34 years age group in England and Wales in an attempt to present an estimate of the incidence of sudden cardiac death and underlying cardiac causes.

Methods

The ONS is the government agency responsible for compiling, analysing, and disseminating many of the UK statistics including periodic census of the population and health statistics.[13] The data used in the mortality statistics are derived from information obtained by the doctor certifying the death, the coroner, and details supplied by the informant to the Registrar. The deaths in males and females are reported at 5-yearly age intervals. The causes of death are registered according to the International Classification of Diseases-10 (ICD-10).[14]

The investigators analysed the ONS mortality data stating the cause of death for England and Wales for four consecutive years 2002–2005, inclusive. Five of the ICD-10 chapters were included in the analysis (*Table 1*). Within these chapters, two of the senior authors (E.R.B. and M.N.S.) scrutinized the ICD-10 classification codes to identify codes that may represent cardiac deaths. Data were then summed from the existing age subgroups to include deaths of individuals from the age of 1 year to the age of 34 years. The selected ICD-10 codes were subsequently divided into four classes as deemed relevant by the investigators (*Table 2*): Class A1: definite cardiac deaths with no structural heart disease identified at post-mortem representing SADS; Class A2: definite cardiac deaths with structural heart disease identified at post-mortem comprising sudden and non-sudden deaths with likely causation by structural heart disease; Class A3: definite cardiac deaths with indeterminate cause comprising sudden and non-sudden deaths where the presence or absence of underlying heart disease was either not recorded or ill defined; and Class B: possible cardiac deaths since a proportion of these deaths may represent misclassifications of cardiac deaths and in particular SADS as epilepsy or drowning. Where there was disagreement relating to the class of an ICD-10 code, a third senior author (S.S.) was consulted. Although the great majority of deaths referred to 'natural causes' (non-accidental, non-malicious), a small proportion of the total cohort is likely to represent accidental deaths since ICD-10 codes W65–W74 from Chapter XX representing accidental drowning and submersion were included in Class B.

Incidence rates were calculated based on the ONS census data of the resident population for individuals aged 1–35 years in England and Wales. Data were further analysed according to age subgroup and gender in order to identify potential trends or gender differences.

Table 1 List of the World Health Organisation international classification of diseases-10 chapters included in the analysis[16]

Chapter VI	Diseases of the nervous system
Chapter IX	Diseases of the circulatory system
Chapter X	Diseases of the respiratory system
Chapter XVIII	Symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified
Chapter XX	External causes of morbidity and mortality

Table 2 Examples of the most frequent International Classification of Diseases-10 codes included in each class (presented in order of frequency)

Class	ICD-10 code
Class A1	R96: other sudden death, cause unknown I49.9: cardiac arrhythmia, unspecified I46.1: sudden cardiac death, so described I45.6: pre-excitation syndrome (WPW)
Class A2	I21.9: acute myocardial infarction, unspecified I25.1: atherosclerotic heart disease I42.0: dilated cardiomyopathy I42.9: cardiomyopathy, unspecified
Class A3	I50.9: heart failure, unspecified I51.9: heart disease, unspecified I50.1: left ventricular failure I50.0: congestive heart failure
Class B	G40.9: epilepsy, unspecified G41.9: status epileptics, unspecified W69: drowning and submersion while in natural water J46: status asthmatics

Statistical analysis

Data manipulation and analysis were undertaken using SPSS software, version 14 (SPSS Inc., Chicago, IL, USA). Data are expressed in means and standard deviations. Annual mortality incidence per 100 000 was calculated as the mean of the 4 years using the following type: (100 000 × number of deaths)/population size. χ^2 or Fisher's exact test was used to test group differences of proportions.

Results

The number of deaths in the 1–34 years age group is reported in *Table 3* according to class and year of death. Analysis of the ONS data revealed an average of 419 (SD 16.5) definite cardiac deaths per annum (Class A1 + A2 + A3) equating to eight young cardiac deaths per week in England and Wales. On the basis of the average estimated size of the resident population of 23 564 050, these data indicate an incidence of young cardiac death of 1.8 (SD 0.08) per 100 000 per year. There were also an average of 433 (SD 6.2) deaths per year, corresponding to more than 8 deaths/week, in Class B which comprised primarily deaths from drowning, epileptic seizures, and other ill-defined causes of

Table 3 Number of deaths according to class per year

Class	Number of deaths per year				Total number of deaths	Mean deaths per annum (SD)	Mean mortality rate per 100 000 per annum (SD)
	2002	2003	2004	2005			
A1	60	50	57	61	228	57.00 (4.97)	0.24 (0.02)
A2	363	358	324	324	1369	342.25 (21.17)	1.45 (0.09)
A3	19	20	21	20	80	20.00 (0.82)	0.09 (0.00)
A1 + A2 + A3	442	428	402	405	1677	419.25 (19.10)	1.78 (0.08)
B	438	424	434	436	1732	433.00 (6.22)	1.84 (0.03)
Total (A1 + A2 + A3 + B)	880	852	836	841	3409	852.25 (19.67)	3.62 (0.08)

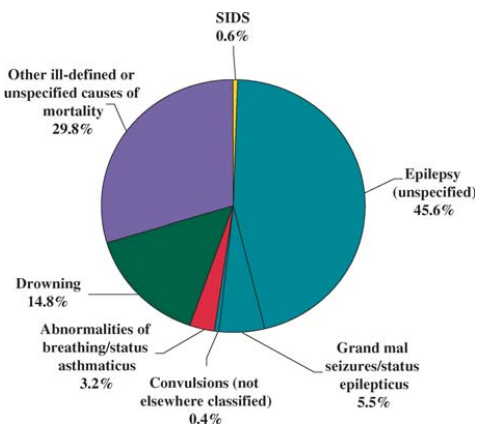

Figure 1 Causes of death in the young expressed as percentage of the total number of deaths in Class B (possible cardiac deaths). SIDS, sudden infant death syndrome.

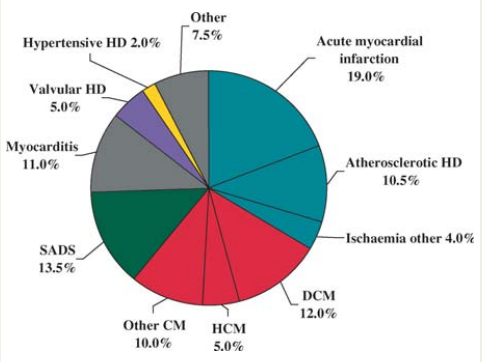

Figure 2 Causes of cardiac death in the young expressed as percentage of the total number of definite cardiac deaths (A1 + A2 + A3). CM, cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HD, heart disease; SADS, sudden arrhythmic death syndrome.

mortality (*Figure 1*). There was no significant variation in the number of the resident population or the number of deaths per year.

The most prevalent causal cardiovascular pathology was ischaemic heart disease comprising one-third (33.5%) of the definite cardiac deaths (A1 + A2 + A3). Although the majority of ischaemic deaths (56%) were attributed to acute myocardial infarction (ICD-10 code: I21.9), in a significant proportion (32%), the presence of atherosclerotic heart disease alone (ICD-10 code: I25.1) was documented as the principal cause of death, comprising 19% and 11% of the definite cardiac deaths, respectively (*Figure 2*). Cardiomyopathies were the second commonest cause of cardiac death corresponding to 27% of definite cardiac deaths in the cohort with dilated and hypertrophic cardiomyopathies accounting for 12% and 5% of the deaths, respectively. Sudden arrhythmic death syndrome accounted for 14% of definite cardiac deaths followed by myocarditis (11%), valvular heart disease (5%), and hypertensive cardiomyopathy (2%) (*Figure 2*).

Causes of death by gender and age

Definite cardiac deaths (Class A1 + A2 + A3) were more prevalent among males with a male to female ratio of 2.4 : 1. The only pathology associated with a statistically significant difference between the male and female gender was ischaemic or potentially ischaemic causes which accounted for 22% of all deaths in males but only 13% in females ($P < 0.001$). The same gender difference was also observed for possible cardiac deaths (Class B) with a male to female ratio of 2.0 : 1. Deaths attributed to epilepsy comprised a greater proportion of female deaths (male vs. female; 22% vs. 31%, $P < 0.001$), whereas drowning-related deaths were more prevalent among males (9% vs. 4%, $P < 0.001$) (*Figure 3*).

There was a rising incidence of definite cardiac deaths with advancing age, with individuals ≥30 years old having a 10-fold risk compared with children aged <10 years. The only underlying causes of definite cardiac deaths exhibiting a significant age trend were ischaemia and cardiomyopathies. Ischaemic deaths exhibited an increasing trend with advancing age, accounting for almost one-third (31%) of all deaths in the 30–34 years age group but

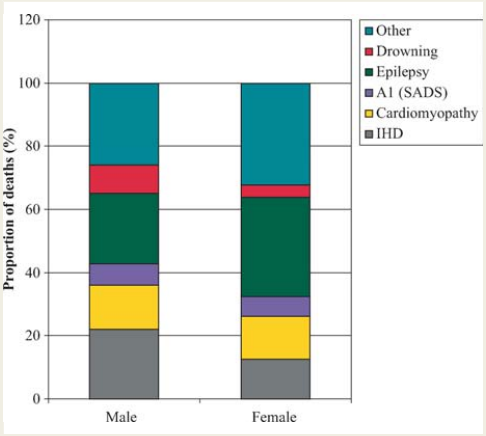

Figure 3 Proportional (%) distribution of underlying cause of death by gender. IHD, ischaemic heart disease; SADS, sudden arrhythmic death syndrome.

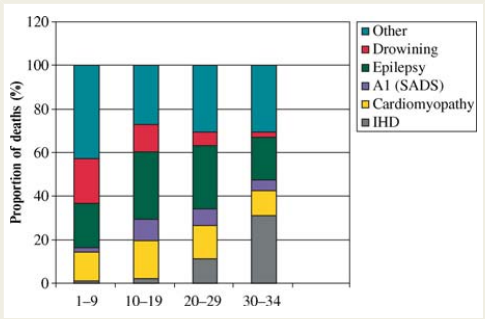

Figure 4 Proportional (%) distribution of underlying cause of death by age. IHD, ischaemic heart disease; SADS, sudden arrhythmic death syndrome.

only 1% in children aged <10 years ($P < 0.001$). In contrast, cardiomyopathy-related deaths peaked during adolescence, accounting for a greater proportion of deaths at the 10–19 years age group (18%) and accounting for only 11% of deaths in the 30–34 years age group ($P = 0.001$) (*Figure 4*).

A similar trend was observed with possible cardiac deaths in Class B, with individuals 30–34 years old having a five-fold risk compared with children aged <10 years old. Underlying causes exhibiting a statistically significant age trend included deaths attributed to epilepsy and drowning. Epilepsy-related deaths peaked in the second and third decades of life, accounting for almost one-third of deaths in the 10–19 and 20–29 years age groups ($P < 0.001$). In contrast, drowning exhibited a reverse trend with age, accounting for 21% of deaths in children aged <10 years but only 3% in individuals ≥30 years old ($P < 0.001$) (*Figure 4*).

Discussion

According to the ONS data, the incidence of cardiac death in the young in England and Wales is 1.8 per 100 000 per year, which corresponds to eight young lives per week. This figure is lower than those documented in retrospective studies of sudden death in the USA in young military recruits and young individuals in Minnesota, which relied on data such as death certificates and post-mortem reports[7,8] and more in keeping with prospective studies in Italy where the reported incidence of sudden cardiac death in young Italian athletes subjected to pre-participation cardiovascular evaluation is 2.1 per 100 000 per year.[9] Therefore, it is likely that our figure derived from the ONS data is a significant underestimate of the true incidence of cardiac death in the young, given the nature of our study and the absence of systematic screening in the UK.

Sudden arrhythmic death syndrome

The incidence of Class A1 deaths that best correlate with SADS was 0.24 per 100 000 per year, with no significant gender or age predilection. This is significantly higher than the previous incidence of 0.10 per 100 000 per year obtained from the ONS statistics for 1997–1999 that used the ICD-9 classification, although an associated prospective national coroner survey would suggest that calculations based on ONS data continue to underestimate the true incidence of SADS.[4] The most plausible explanation for this discrepancy is that the ONS mortality statistics are derived largely from documentation on death certificates which may under-report the true incidence of cardiac arrhythmias. Indeed the classification of deaths by ONS does not differentiate sudden from non-sudden deaths clearly, although most of the pathologies reported as causes of deaths, such as atherosclerotic heart disease and cardiomyopathy, would be more likely to cause sudden rather than non-sudden death in this age group. Malignant cardiac arrhythmias secondary to ion channelopathies such as Brugada syndrome and long-QT syndrome may manifest as epileptiform seizures and collapse secondary to brain anoxia or drowning resulting in misclassification of genuine cases of SADS as epilepsy[15,16] or unexplained drowning.[17,18] The latter is particularly relevant since there is a well established association between the most common subtype of long-QT syndrome, LQT1, and sudden death in swimmers.[19,20] Conversely, post-mortem findings such as coronary atherosclerosis without significant narrowing of the arterial lumen or without macroscopic or microscopic evidence suggestive of acute or chronic ischaemia, as well as mitral valve prolapse and myocarditis,[21] are quite common and may be falsely attributed as the cause of death.

Mortality gender and age predilection in the young

Consistent with prior literature reports, ischaemic or potentially ischaemic causes contributed a greater proportion of deaths in males and with increasing age, in particular after the age of 30 years. Conversely, potentially inherited cardiomyopathies such as hypertrophic and dilated cardiomyopathies did not exhibit any gender predilection but there was a significant age trend,

contributing a greater proportion of deaths in the 10–19 age group with a gradual decrease thereafter.

Epilepsy appeared to exhibit a female predilection, accounting for a greater proportion of deaths during adolescence. These results should however be viewed with caution given the limited data available and the multiple factors which may influence epilepsy-related mortality, as established by a prior large, prospective study in the UK.[22] Finally, in accord with previous reports, drowning-associated deaths were more prevalent among males and children aged <10 years, with a reverse trend with increasing age.[23]

Clinical implications

Considering the devastating impact of sudden and cardiac death in the young and the potential number of life years lost our findings suggest that the number of deaths identified (8 deaths/week) is sufficiently high to command attention even without the inclusion of potential misclassifications (Class B). If consideration is given to the possibility that at least 20% of deaths attributed to epilepsy or drowning may actually be caused by a primary myocardial electrical disorder, then the estimate of sudden and cardiac death in the young is at least 10 per week.

It is imperative that awareness of young cardiac deaths and in particular SADS is raised among pathologists and coroners to ensure that accurate conclusions are derived from autopsies. If the autopsy identifies cardiomyopathy or a case of SADS, this should trigger the referral of families of victims for comprehensive cardiological screening and guide their assessment. This is important because a significant proportion of conditions implicated in young cardiac death and SADS, in particular, are inherited. Indeed, over half of families with SADS deaths demonstrate evidence of an ion-channel disorder or a cardiomyopathy.[5,6] Reforms and further tightening of procedures to address these issues have been proposed, and a national pathology registry has been launched in the UK.[24,25]

Increased awareness among primary care physicians is also vital to ensure recognition of cardiac conditions in young people with the propensity to cause sudden cardiac death since these deaths are often preceded by warning symptoms including syncope. Appropriate assessment may therefore prevent a proportion of these tragic deaths.[6]

Limitations

This epidemiological study exhibits some important limitations that warrant mention. The cause of death was ascertained from the ONS data and the authors did not examine death certificates or post-mortem reports on an individual basis in order to identify potential misclassifications. Although the ONS data do not provide information regarding the number of deceased individuals who underwent a post-mortem examination, it would be reasonable to assume that the majority of the victims were subjected to a post-mortem examination given their youth and the UK medico-legal implications.

The investigators concede that this study relied solely on information provided to the ONS from documentation on death certificates and post-mortem reports which may not always accurately reflect the true cause of death given the ambiguities related to the diagnosis of conditions associated with sudden cardiac death in the young. This may explain the absence of conditions such as arrhythmogenic right ventricular cardiomyopathy (ARVC) as a distinct entity, whereas minor manifestation of certain common disorders such as atherosclerosis may have been falsely attributed as the cause of death. In addition, ARVC does not have its own ICD-10 code and is classified currently as I42.8—other cardiomyopathies. The purpose of this study, however, was to provide an estimate of the incidence of cardiac death and underlying cardiac causes in the young in order to highlight the need to establish the scale and nature of the problem.

Contributors

M.P., S.S., S.C., M.N.S., V.F.P., and E.R.B. carried out the literature search, collected, analysed, and interpreted the data, drafted the article and revised it critically for scientific content, and approved the final version for publication. E.R.B. is the guarantor.

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Aetiology of sudden cardiac death in athletes in the United Kingdom: a pathological study

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ABSTRACT

Objective: To characterise the demographics and aetiology of sudden cardiac death (SCD) in athletes referred to a tertiary cardiac pathology centre in the UK.

Design: Retrospective non-case controlled analysis.

Setting: Cardiac pathology centre at the National Heart and Lung Institute and Royal Brompton Hospital.

Subjects: Between 1996 and 2008, the hearts of 118 athletes were referred for pathological assessment to ascertain the precise aetiology of SCD.

Results: The majority of athletes (n = 113; 96%) were male and most (107; 91%) were amateurs participating predominantly in football, rugby and running. The mean (SD) age of death was 28 (12) years (range 7–59); 75% athletes were aged ⩽35 years. Most deaths (81%) occurred during or immediately after exercise. Antecedent symptoms of cardiac disease were reported in 21 (18%) subjects, and 20 (17%) had a family history of premature cardiovascular disease and/or SCD. 25 (21%) athletes had relevant past medical history which included a known history of cardiac disease. Cardiomyopathy was the commonest cause of death and accounted for 62% of all the SCDs. A significantly high proportion of athletes (23%) exhibited a morphologically normal heart. Atherosclerotic coronary disease accounted for only 3% of cases and was confined to athletes aged >35 years.

Conclusions: SCD in sport is largely due to clinically silent cardiomyopathies or primary electrical disorders (morphologically normal heart). Antecedent symptoms and family history are absent in over 80% of cases, and therefore clinical screening with health questionnaires will fail to identify most athletes with potentially sinister cardiac disorders.

The sudden death of an athlete is a rare event with an incidence ranging between 1:50 000[1] to 1:200 000[2] a year. However, such events are highly publicised and have a substantial emotional impact on the community at large when one considers that athletes are perceived as the healthiest segment of society.

Over 80% of non-traumatic-exercise related deaths are attributable to cardiac disorders.[1–5] The majority of sudden cardiac deaths (SCDs) in young athletes (<35 years) are due to hereditary or congenital cardiac anomalies; hypertrophic cardiomyopathy (HCM) is reported to be the commonest cause of death in young athletes world wide. In contrast, the vast majority of SCDs in older athletes are secondary to atherosclerotic coronary artery disease.

Most datasets examining SCD in athletes are derived from American[3 5] and Italian[6 7] studies. The United Kingdom lacks a national registry for systematically reporting sudden death in sports, and therefore knowledge relating to antecedent warning symptoms, the demographics of victims, circumstances and prevalent causes of sudden death in athletes in a sizeable cohort is lacking. However, such information is fundamental to facilitate any debate about local provisions for a potential pre-participation cardiovascular screening programme and subsequent exercise recommendations.[8–10]

The aim of this study was to identify the characteristics and causes of death in a large cohort of athletes referred to the Royal Brompton Hospital, a specialist tertiary cardiac pathology centre in the UK.

METHODS

Between January 1996 and July 2008, 118 cases of sudden death in people participating in regular sport activities were referred to the Cardiac Risk in the Young (CRY) Centre for Cardiac Pathology at the Royal Brompton Hospital for further evaluation, by coroners and pathologists throughout the UK.

Definitions

The term "sudden death" was defined as sudden unexpected death (within 1–12 h of apparent wellbeing) from natural causes during or shortly after (within 24 h) exercise. The individual was considered an "athlete" if he or she participated in at least 2 h/week of organised physical training and competed in regular team or individual sport.

Subjects

Subjects were divided into two groups based upon their age at death: (*a*) ⩽35 years and (*b*) >35 years. Data on age, sex, circumstances of death, sporting discipline, antecedent cardiac symptoms, past medical history and a family history of cardiac disease (when available) of the deceased subject were obtained from the referring pathologist or coroner.

Toxicology screen

All patients included in this study underwent a toxicology screen as part of the coroner's mandate since all deaths were sudden and unexpected.

Pathological analysis

Pathological analysis of all hearts was performed by the senior author (MNS) with the consent of the coroner and family of the deceased. The heart weight was recorded and measurements of the left and right ventricular wall thickness and internal cavity dimensions were made at mid-ventricular level excluding papillary muscle and fat. Sections of

myocardium were fixed in formalin, embedded in paraffin and stained with haematoxylin and eosin as well as elastic Van Gieson stain to highlight myocardial fibrosis.

The extramural coronary arteries were studied macroscopically in the intact heart by making multiple cross sections of the vessels (3–5 mm apart). Table 1 gives the macroscopic and histological criteria for specific cardiac diseases.

Results were reported in four broad categories: (*a*) cardiomyopathies; (*b*) coronary artery pathology; (*c*) morphologically normal heart (*d*) and other cardiac pathology.

Statistical analysis

Means and standard deviations (SD) were calculated for continuous variables. Data were compared with Student's *t* test where appropriate.

RESULTS

Demographics

Of the 118 cases of SCD, the majority were amateur sportsmen (n = 107, 91%) and included seven subjects who had participated in 2–23 marathons. The remaining 11 cases (9%) were seven athletes (6%) at a professional or semiprofessional level (soccer n = 6, cycling n = 1) and four (3%) who participated in intensive physical training in the armed forces.

The subjects were predominately male (n = 113; 96%). One hundred and thirteen athletes (96%) were white and five were black (African/Caribbean in origin). The mean (SD) age of SCD in this series was 27.9 (12.5) years (range 7–59). Seventy-five per cent of all deaths were in subjects aged ⩽35 years and almost one-third were in child or adolescent athletes (<18 years). The greatest number of deaths (n = 20) occurred in the 16–20 year age group (fig 1). With the exception of one case, all female athletes who died were in the younger age group.

The vast majority of SCDs (81%) occurred during (66%) or immediately after (15%) exercise. In relation to sporting discipline, most deaths occurred in soccer (n = 44; 37%; age 11–35 years), followed by running (n = 24; 20%; age 8–59 years) and rugby (n = 11 cases; 9%, age 15–42 years) (table 2).

Antecedent symptoms, past medical history and family history of cardiac disease

Of the 118 cases, 21 (18%), had experienced one of more antecedent symptoms suggestive of underlying cardiac disease (table 3).

In 20 subjects (17%) there was a family history of premature cardiovascular disease (with the majority of cases comprising ischaemic heart disease, 69%) and/or family history of SCD (⩽50 years old) in a first-degree relative.

Twenty-five (21%) of the subjects had relevant previous medical history. Seven subjects had a history of cardiac disease, nine were clinically suspected to have underlying cardiac pathology, five had risk factors for coronary artery disease and four had had at least one epileptic seizure (table 3). Ten athletes (8%) also had a previous diagnosis of asthma. As far as the authors can ascertain, none of the asymptomatic subjects had been subject to pre-participation cardiovascular evaluation to identify disorders capable of causing SCD.

Causes of sudden cardiac death

Abnormal cardiac pathology (macroscopic and/or microscopic) was identified in 91 (77%) of all cases. The remaining subjects had a morphologically normal heart (table 4 and fig 2).

Toxicology screen

All but two subjects had a normal toxicology screen. Both were body builders and had blood traces of anabolic steroids.

Table 1 Macroscopic and microscopic criteria for determining cardiac pathology

Disorder	Macroscopic appearance	Microscopic appearance
Cardiomyopathy		
Hypertrophic cardiomyopathy	Left ventricular wall thickness ⩾15 mm and or heart weight ⩾500 g	Myocyte hypertrophy, disarray and interstitial fibrosis
Idiopathic left ventricular hypertrophy	Left ventricular wall thickness ⩾15 mm and or heart weight ⩾500 g	Myocyte hypertrophy with or without fibrosis and no myocyte disarray
Arrhythmogenic right ventricular hypertrophy	Right ventricular thinning with fatty replacement and fibrosis	Fat and fibrosis throughout the wall of the right ventricle and/or left ventricle
Idiopathic fibrosis	Normal left ventricular thickness ⩽15 mm	Focal and diffuse interstitial and/or replacement fibrosis in the ventricular wall
Coronary artery pathology		
Atherosclerosis	Atherosclerosis with narrowing >75%	Acute/chronic infarction in the left ventricle
Anomalous coronary artery	Anomalous origin of the coronary artery, coronary artery atresia, stenosis	Fibrosis/acute/chronic infarction in the left ventricle
Coronary artery spasm	Circumferential subendocardial haemorrhagic infarction with normal coronaries	Acute infarction in the left ventricle
Coronary dissection	Tear in the coronary artery media	Infarction in territory of artery
Morphologically normal heart	Normal	Normal
Other cardiac pathology		
Myocarditis	Normal, dilated wall	Inflammation with myocyte necrosis
Floppy mitral valve	Mitral valve ballooning	Myxoid change in the valve
Sickle cell crisis	Normal	Sickle cell in intramural coronary vessels with microinfarcts in the myocardium

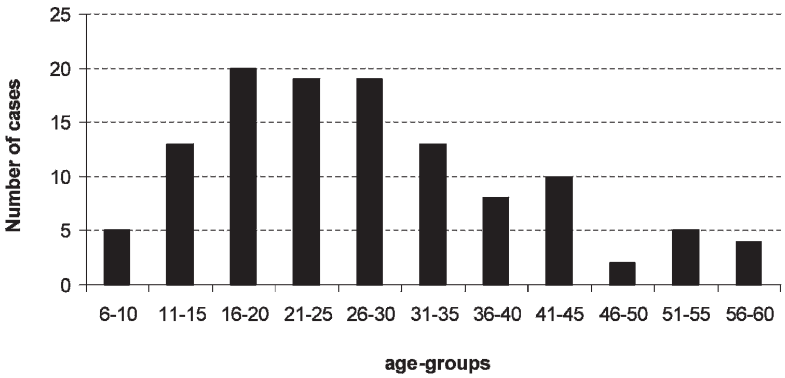

Figure 1 Bar chart showing the number of sudden deaths in athletes in relation to age in 118 deaths in sportsmen referred to a tertiary centre in the UK over a 12-year period.

Cardiomyopathy

Deaths attributed to a primary myocardial disorder (cardiomyopathy) were identified in 73 (62%) of all athletes with an SCD. Left ventricular hypertrophy (LVH) was the most commonly identified abnormality on macroscopic examination and was detected in 49 (42%) athletes raising the possibility of HCM. However, only 13/49 (27%) athletes with LVH exhibited associated myocyte disarray, the historically regarded histological hallmark of HCM. The remaining cases of LVH (n = 36; 31%) were associated with histological evidence of either isolated myocyte hypertrophy (n = 27) or myocyte hypertrophy and fibrosis (n = 9). The authors classified these cases under the term "idiopathic LVH". One case of HCM and another of idiopathic LVH was associated with the presence of anabolic steroid traces on toxicology screen.

Athletes with HCM were predominantly in the younger age group (mean (SD) age 24.6 (7.1); range 11–43) and all but one case was male. Similarly, 71% of all victims with idiopathic LVH were in the younger age group (mean (SD) age 32.7 (12.6) years; range 9–59) and all were male. Of the five Afro-Caribbean subjects, four had evidence of idiopathic LVH.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) was the second most common diagnosis and was identified in 16 (14%) members of our cohort. There was evidence of biventricular involvement in 50% of cases. In contrast with cases of idiopathic LVH and HCM, deaths were equally distributed between the older and younger age group (mean (SD) age 35.9 (12.2) years; range 16–57).

Table 2 Demographic characteristics of the cohort

Characteristics	Values
Number of subjects	118
Male	113 (96)
Age (years), mean (SD), {range}	27.9 (12.5), {7–59}
Number of subjects ⩽35 years	89 (75)
Sport discipline	
Soccer	44 (37)
Running	24 (20)
Rugby	11 (9)
Cycling	8 (7)
Swimming	5 (4)
Weight lifting	3 (3)
Golf	3 (3)
Other (⩽2 subjects/discipline)	20 (17)

Results are shown as number (%) unless stated otherwise.

Idiopathic fibrosis without LVH occurred in seven cases with all but one in the younger age group (mean (SD) age 28.4 (8.5) years; range 17–43). Finally, one case exhibited features of both arrhythmogenic right ventricular cardiomyopathy and HCM. There was insufficient tissue sampling to provide a definite diagnosis and the case was classified as an undetermined cardiomyopathy.

Coronary artery pathology

Coronary artery pathology was identified in 11 (9%) subjects. The main pathology was a congenital anomaly of the coronary arteries which was seen in six of the 11 subjects all of whom were male and ⩽35 years old (mean (SD) age 15.8 (6.2) years; range 7–25). Both coronary arteries arose from the same coronary ostium in four cases; two cases had the left coronary artery arising from the right sinus and two cases had the right

Table 3 Antecedent symptoms, family history and relevant cardiac history of the 118 subjects*

Patient information	No of cases	Age (years) ⩽35	Age (years) >35
Family history	*20 (17%)*	*15*	*5*
Family history of heart disease	13	9	4
Family history of SCD	8	6	2
Symptoms	*21 (18%)*	*16*	*5*
Syncope/dizziness	10	9	1
Shortness of breath	7	5	2
Palpitations	6	6	0
Chest pain	4	3	1
Relevant past medical history	*25 (21%)*	*16*	*9*
Clinical suspicion of cardiac disease after investigation†	9	6	3
Epilepsy/seizure	4	4	0
Heart murmur	3	3	0
Diabetes	3	1	2
Hypercholesterolaemia	4	0	4
Congenital heart disease	1	1	0
Ventricular septal defect	2	2	0
Previous cardiac arrest	2	1	1
Atrial fibrillation/pacemaker insertion	1	0	1
Viral myocarditis	1	1	0

*Symptoms/findings do not add up since some subjects had more than one symptom/finding; †abnormal ECG (n = 4), abnormal echo (n = 2), possible arrhythmia (n = 3). SCD, sudden cardiac death.

Table 4 Cause of sudden cardiac death according to histopathological findings

Diagnoses	⩽35	>35	Total
Cardiomyopathy	*49*	*24*	*73*
Idiopathic left ventricular hypertrophy	19	8	27
Idiopathic left ventricular hypertrophy with fibrosis	3	6	9
Arrhythmogenic right ventricular cardiomyopathy	9	7	16
Hypertrophic cardiomyopathy	11	2	13
Idiopathic fibrosis	6	1	7
Undetermined cardiomyopathy	1	0	1
Morphologically normal heart	*26*	*1*	*27*
Coronary artery pathology	*7*	*4*	*11*
Anomalous coronary artery	6	0	6
Atherosclerosis	0	3	3
Coronary "spasm"	1	0	1
Coronary dissection	0	1	1
Other cardiac pathology	*7*	*0*	*7*
Myocarditis	3	0	3
Floppy mitral valve	2	0	2
Complex congenital heart disease	1	0	1
Sickle cell crisis	1	0	1

coronary artery arising from the left sinus. Of the remaining two cases, one had atresia and hypoplasia of the left coronary artery and the other stenosis of the ostium and a shelf-like slit of the left coronary artery. Coronary atherosclerosis was the cause of death in only three athletes who were all aged >35 years (mean (SD) age 49.7 (4.0) years; range 45–52). A single case of coronary artery spasm was detected in a 17-year-old man, as well as one case of spontaneous dissection of the left anterior descending artery in a 38-year-old man.

Other cardiac pathology

Lymphocyte myocarditis was the predominant finding in three cases. Two subjects had valvular disease which included floppy mitral valve and associated myocardial fibrosis. There was a single subject with corrected, complex congenital heart disease of univentricular circulation and Fontan circulation who exhibited biventricular hypertrophy with no other associated anomalies. Finally, one subject had evidence of an acute sickle cell crisis with sickling within the coronary arteries and associated acute ischaemia.

Morphologically normal heart

In almost a quarter of our cohort (23%) the post mortem disclosed no cardiac abnormality which could account for the cause of death, despite detailed macroscopic and microscopic examination. The majority of these cases (96%) were in the younger age group where the mean (SD) age of 18 (6.1) years (range 8–42) was significantly lower than that of those dying with identifiable cardiac pathology ($p<0.001$). Interestingly, three of the five swimmers who died had a morphologically normal heart.

Female athletes

Of the five females athletes in the series, two had a morphologically normal heart, one had HCM, one exhibited idiopathic fibrosis and another, coronary atherosclerosis.

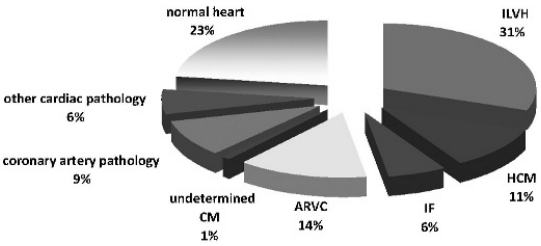

Figure 2 Pie chart showing the causes of sudden cardiac death in 118 sports deaths referred to a tertiary cardiac centre in the UK over 12 years. ARVC, arrhythmogenic right ventricular cardiomyopathy; CM, cardiomyopathy; HCM, hypertrophic cardiomyopathy; IF, idiopathic fibrosis; ILVH, idiopathic left ventricular hypertrophy. Percentages do not add up to 100% because of rounding.

DISCUSSION

The cardiovascular benefits of regular physical activity are well established[11] and only a small proportion of athletes with unsuspected cardiac pathology are at increased risk of exercise-related SCD.[1–7] The majority of data examining the aetiology of deaths in athletes originates from the USA (1866 cases (whole series), 690 cases are primary CVD only)[5] and Italy (55 cases),[7] although a number of small studies also exist in other European countries, including Spain (61 cases),[12] France (80 cases)[13] and Ireland (51 cases).[14] Data in the UK are scarce and limited to a small group of older sport participants.[14–16] As far as we know, this study of 118 SCDs in athletes is the largest reported series in the UK.

Sport and gender predilection

Consistent with large American[5 17] and Italian[6 7] series of SCD in athletes, just over 80% of deaths occurred during or immediately after exercise, indicating that the interplay of physical, metabolic and endocrine stresses of exercise on the heart is an important trigger for fatal ventricular arrhythmias. Soccer and running were the sports most commonly associated with SCD and this is in agreement with most other studies.[1–7 10] This sport bias most certainly represents the high participation rates in these sporting disciplines in most Western European countries. In concurrence with previous studies, the great majority (96%) of subjects with SCD subjects in our cohort were male.[1–7 12–17] This may be attributed to the lower participation rate of women in sport generally and specifically in sports popular with male subjects, such as soccer, which is the predominant sport in our study.

Cardiomyopathy

Consistent with previous studies in the USA[2 3 5 17] and Italy,[1 6 7] our results indicate that cardiomyopathies are the most prevalent underlying pathology in SCD related to athletic activity. In contrast, however, in this series LVH without myocyte disarray, was the predominant finding (31%), compared with HCM and ARVC in the USA and Italy, respectively.

Idiopathic LVH is becoming increasingly recognised and although it has been reported in previous studies, this is the first series in which it predominates. It is unclear at this stage whether it represents an acquired pathological variant of the physiological LVH exhibited as part of the "athlete's heart" in certain genetically predisposed backgrounds.[18] The finding of idiopathic LVH in four out of five Afro-Caribbean cases of SCD may be relevant in this regard since a recent study in highly trained black athletes has shown that 3% exhibit substantial

LVH (≥15 mm) and it is plausible that in such athletes, marked LVH predisposes to exercise-related fatal ventricular arrhythmias.[19] LVH has also been associated with the use of anabolic steroids.[20] In our study traces of anabolic steroids were identified at post mortem in two body builders; one was diagnosed with idiopathic LVH and the other with HCM.

Idiopathic myocardial fibrosis with or without LVH, featured in 14% of this cohort in contrast to significantly lower rates in previous studies (2%–3%).[12–14] The aetiology and importance of cardiac fibrosis remains unclear; however, transient myocardial damage has been detected in athletes in the post-race setting and has been associated with transient diastolic and systolic dysfunction.[21] Possibly, in some athletes prolonged arduous physical activity may result in myocardial necrosis and subsequent fibrosis. This pathology may represent an acquired, exercise-related cardiomyopathy and/or genetic predisposition leading to a fatal arrhythmia. This concern was raised in a recent case report from our group, a marathon runner who died during a race with marked LVH and myocardial fibrosis.[22] It is also possible that at least some of these cases may be due to the recently recognised familial arrhythmogenic left ventricular cardiomyopathy.[23]

ARVC was the second most common cardiomyopathy and accounted for 14% of all sudden deaths in our series. Our figures were significantly higher than those reported in the US series[2 3 5 17] and not dissimilar from the reports from the Veneto region of Italy[1 6 7 24] and other European countries.[12 13 25–28] These observations suggest that there may be a higher genetic cluster of ARVC in Europe or, owing to the Venetian experience, a greater awareness of this disorder amongst pathologists in Europe.

Of note, we did not observe deaths from dilated cardiomyopathy in our cohort, which contrasts with series from other countries where the range was 2–11%.[1 3 12 13]

Coronary artery pathology

Coronary artery pathology was less prevalent in this study than in the US and Italian experience. Sudden cardiac death secondary to anomalous coronary arteries was confined to the younger age group (median 17 years) in this series, a trend supported by previous reports.[2 3 5–7 12 13 17] Atherosclerotic coronary artery disease was seen in a much smaller number of patients and was confined to the older (>35 years) age group as observed in previous sports deaths series.[14–16]

Morphologically normal heart

In this study there was a high prevalence of SCDs in subjects with a morphologically normal heart (23%) despite detailed macroscopic and histological examination. Previous studies have reported significantly lower rates, as low as 1%,[1 3 5 17] with only a Spanish series reporting a figure comparable to our study (16.3%).[12] The identification of a morphologically normal heart is of great importance since studies in the USA and the UK suggest that more than 50% of such SCDs are caused by malignant arrhythmias secondary to the presence of inherited ion channel disorders usually affecting the potassium, sodium and calcium ion channels of the myocardial cells.[29 30] A previous study undertaken at our unit highlights the prominent role of electrical abnormalities in the normal heart in SCD, particularly in younger subjects.[31]

Although selection bias has certainly contributed to the high prevalence of SCD with a morphologically normal heart, this study highlights the importance of establishing such a diagnosis since ion channelopathies can be identified in other relatives with non-invasive cardiac investigation, and appropriate treatment instigated to avoid further tragedies.[32] Of interest, three children (aged 7–15 years) with morphologically normal hearts died during swimming. This sport has been particularly associated with deaths in the long Q–T syndrome[33 34] and catecholaminergic polymorphic ventricular tachycardia.[35]

Clinical implications related to pre-participation screening

Sudden death is often the first clinical manifestation of underlying heart disease in young athletes. The Italian model of pre-participation cardiovascular screening in young athletes, using 12-lead ECG as a screening tool, is effective in reducing SCD by identifying athletes with underlying cardiomyopathy and ion channelopathies.[6 7] This study indicates that based on the high prevalence of cardiomyopathies and the relatively low occurrence of coronary artery pathology in our subjects, pre-participation cardiovascular screening using a 12-lead ECG would probably have detected the underlying cardiac abnormality in a significant number of SCD victims. This argument is further reinforced by the high prevalence of SCDs in people with a morphologically normal heart, of which a significant proportion may be attributed to inherited ion channelopathies and which potentially might be detected by the 12-lead ECG.

The absence of antecedent cardiac symptoms and/or a family history of cardiac disease and/or SCD in almost 80% of cases of SCD confirms the prior observation that most cardiovascular disorders responsible for SCD in the athlete are clinically silent and unlikely to be discovered from spontaneous symptoms. Cardiovascular screening including ECG testing will be associated with a significantly higher diagnostic yield than reliance on history alone.[28 36–38]

We recognise that almost one-third of the cases in this series exhibited idiopathic LVH and it may argued that these cases may not have been identified with ECG. However, it is unclear at this stage whether idiopathic LVH represents a spectrum of the HCM phenotype, an exaggeration of the physiological response[18] resulting in pathological LVH or whether it is indeed an innocent bystander (genuine physiological LVH) in a person who may have succumbed to a fatal ventricular disorder owing to an undetectable ion channel disorder or congenital accessory pathway. However, it is well established that over 90% of people with HCM have an abnormal ECG.[39] The experience of two of the authors (SS and GW) of screening over 3000 highly trained British athletes aged 14–35 years identified three athletes who might have been considered to have HCM; all three exhibited grossly abnormal ECGs.[40] Although ion channel disorders cannot be identified and accessory pathways may be difficult to demonstrate at autopsy, ante mortem 12-lead ECG has a high yield in the identification of ion channel disorders[32] and accessory pathways. Based on these facts we suspect that a significant proportion of athletes with idiopathic LVH would have been identified on a routine ECG and in an expert setting would have been subject to further investigations aimed at confirming the pathological diagnosis and the assessment of risk of SCD.

The predominance of deaths due to idiopathic LVH and idiopathic myocardial fibrosis does raises the possibility that some deaths in sport may be secondary to acquired myocardial disorders resulting from the long-term effects of intensive exercise,[18] warranting several cardiac assessments throughout the athlete's career.

The Italian screening programme in athletes has been successful in identifying and preventing deaths predominantly

from the cardiomyopathies through subsequent disqualification of the affected subject from sporting activities of moderate to high intensity to minimise the risk of SCD. In this regard it is prudent to highlight that in this study almost one-fifth of all SCDs in athletes occurred at rest, suggesting that the identification of cardiovascular diseases and subsequent disqualification from sport will not prevent deaths in all athletes harbouring potentially fatal cardiac disorders.

Limitations

We concede that conclusions drawn from this study do not necessarily apply to the whole of the UK because of a significant selection bias. The CRY Centre for Cardiac Pathology at the Royal Brompton Hospital is an internationally recognised cardiac pathology centre in the UK, where the hearts of many young athletes are commonly referred, especially when the findings are ambiguous and no clear cause of death can be established by the local pathologist. It is therefore highly probable that cardiac anomalies such as coronary artery atherosclerosis, which can be easily identified, and cardiomyopathies such as HCM, which have been well characterised, are under-represented in this cohort. Similarly the prevalence of less well-defined entities such as idiopathic left ventricular hypertrophy and a morphologically normal heart are likely to represent an overestimate. A national database on SCD in young athletes needs to be established in order to obtain more accurate information in the future.

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Authorship and contributorship: SN, conception and design, literature search, analysis and interpretation of data and drafting the article; SS, SD, MP, GW, MNS, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content; MNS, additionally, performed post mortems. All the authors approved the final version to be published.

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CLINICAL RESEARCH

The importance of specialist cardiac histopathological examination in the investigation of young sudden cardiac deaths

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Aims Post-mortem examination of the heart in young sudden cardiac death (SCD) is vital as the underlying aetiology is often an inherited cardiac disease with implications for surviving relatives. Our aim is to demonstrate the improvement in diagnostic quality offered by a specialist cardiac pathology service established to investigate SCD with fast-track reporting on hearts sent by pathologists in cases of SCD.

Methods and results A tertiary centre prospective observational study was conducted. Detailed histopathological examination was performed in a tertiary centre specialized in the investigation of cardiac pathology in SCD. Hearts from 720 consecutive cases of SCD referred by coroners and pathologists from 2007 to 2009 were included. A comparison was drawn with diagnoses from referring pathologists. Most SCDs occurred in males (66%), with the median age being 32 years. The majority (57%) of deaths occurred at home. The main diagnoses were a morphologically normal heart ($n = 321$; 45%), cardiomyopathy ($n = 207$, 29%), and coronary artery pathology ($n = 71$; 10%). In 158 out of a sample of 200 consecutive cases, a cardiac examination was also performed by the referring pathologist with a disparity in diagnosis in 41% of the cases ($\kappa = 0.48$). Referring pathologists were more inclined to diagnose cardiomyopathy than normality with only 50 out of 80 (63%) normal hearts being described correctly.

Conclusion Expert cardiac pathology improves the accuracy of coronial post-mortem diagnoses in young SCD. This is important as the majority of cases may be due to inherited cardiac diseases and the autopsy guides the appropriate cardiological evaluation of blood relatives for their risk of sudden death.

Keywords Autopsy • Cardiomyopathy • Morphologically normal heart • Sudden arrhythmic death syndrome • Sudden cardiac death • Young

Introduction

Inherited cardiac diseases are common causes of young sudden cardiac death (SCD) and the family must not only contend with the grief of their loss but also with the possibility that SCD may strike the family again.[1] The UK reports at least 10 cases of SCD occurring per week or 1.8 per 100 000 persons-year, with several cases misclassified under drowning or epilepsy.[2] In the UK, national guidelines have outlined the need for specialist cardiological evaluation for families who have suffered an unexplained SCD (sudden arrhythmic death syndrome, SADS) and expert post-mortem examination is a critical first diagnostic step required to guide clinical evaluation of surviving relatives.[3] This aims to identify individuals at the risk of SCD, in whom medical intervention is likely to prevent further tragedies.[1,4–6]

Best practice guidelines set by the Royal College of Pathologists and the Association for European Cardiovascular Pathology recommend referral of whole hearts to specialist centres with high volume and recognized expertise.[7–9] In the UK, all sudden unexpected

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What's new?

- The pathological investigation of sudden cardiac death (SCD) poses significant diagnostic challenges, particularly in the young where inherited cardiac diseases predominate.
- There is a considerable variability in the interpretation of autopsy findings between general and specialist cardiac pathologists, which can lead to alternative diagnoses in 40% of the cases.
- General pathologists are likely to overestimate the significance of autopsy findings and attribute deaths to cardiomyopathy at the expense of diagnosing a morphologically normal heart.
- Isolated left ventricular hypertrophy is present in a considerable proportion of SCDs and its significance remains unclear, warranting further research.
- Investigation of SCD by an experienced cardiac pathologist is essential when an inherited cause is suspected, to ensure diagnostic accuracy, given the potential implications for surviving relatives.

deaths have an autopsy carried out by a pathologist, whereas in the rest of Europe there is widespread variation as to the rate of autopsies for SCD even within the same country.[10] Most British pathologists have limited exposure to cases of young SCD as well as few resources and time constraints to perform the autopsy.[11,12] Moreover, the introduction of the Human Tissue Act in 2004 has limited preservation of tissue at autopsy.[13] All these factors translate to either the absence or the limited histopathological examination in the cases of SCD, translating to an autopsy of reduced and/or variable quality[12,14,15] and may lead to erroneous diagnoses.[2,16] A recent report, which compared autopsy evaluation of SCD in the young before and after best practice guidelines were introduced, determined that the histological analysis of the right ventricular (RV) and left ventricular (LV) tissue was still unsatisfactory with $<$50% of cases providing a histological description of these sites.[17]

The charity Cardiac Risk in the Young (CRY) launched a Centre for Cardiac Pathology, CRY CCP, established in the National Lung and Heart Institute (Imperial College) and Royal Brompton Hospital (RBH) in March 2007 to address the need for detailed histopathological evaluation of cases of SCD from potentially inherited conditions by an expert cardiac pathologist at no cost to the family, coroner, or health service. We report the diagnostic results of this innovative service.

Methods

Population inclusion and exclusion criteria

This study was completed with ethical approval from the Brompton, Harefield and National Heart and Lung Institute: Ref 07/Q040. A total of 753 cases, referred between March 2007 and December 2009 for expert cardiac pathological assessment to the CRY CCP service, were considered for this prospective observational study. Inclusion criteria were as follows: referral by a coronial pathologist of a witnessed instantaneous death; or a suspected sudden death when the individual was seen alive and well up to 24 h prior to death; and where non-cardiac causes had been excluded at initial autopsy. All ages were included to demonstrate the wide age range of referrals received at the centre.

Cases were excluded from the study if the death occurred in the context of deteriorating heart failure ($n = 26$), was non-sudden ($n = 4$), or if subsequent toxicology provided an explanation for the death ($n = 3$). Data on age, sex, and location/circumstances of death of the deceased were obtained from the referring pathologist or coroner. Subjects were divided into two groups based upon their age at death: (i) $\leq$35 years and (ii) $>$35 years.

Specimen referral process

A specific protocol was established for handling an SCD referral and is summarized in *Figure 1*.

Referrals

The total numbers of SCD referrals were calculated before and after the launch of the CRY CCP in March 2007. To examine trends over time, SCD referrals were grouped into two time bands: 1999–2006 and 2007–09.

Heart specimen handling

Sudden cardiac death victims were examined by local pathologists in 45 counties within the UK. Following the exclusion of extra-cardiac causes for the death, the hearts were referred to CRY CCP with the consent of the coroner and the family of the deceased. Established macroscopic and histological criteria for the diagnosis of cardiac pathology were used and are summarized in Supplementary material online, *Table S1*[7–9] For all cases, observations and dimensions were consistently recorded. Ten to twenty tissue sections were routinely taken according to agreed national[9] and international criteria[7] and included: the RV outflow tract; a right lateral cut containing right atrium, posterior leaflet of the tricuspid valve, and lateral RV; a left lateral cut containing left atrium, mitral valve and lateral LV; circumferential RV and LV samples; the anterior and posterior septum; the three major coronary arteries; the ascending aorta; and the conduction system. Extra tissue sections were taken to confirm pathology when this was detected macroscopically and/or microscopically. Sections were fixed in formalin, embedded in paraffin, and stained with haematoxylin and eosin stain or elastic Van Gieson to highlight myocardial fibrosis.

The results were reported in categories: (i) morphologically normal heart, (ii) cardiomyopathies, (iii) coronary artery pathology, (iv) complex congenital heart disease (CHD), (v) inflammatory disease, (vi) valve disease, (vii) aortic disease, (viii) tumour, and (ix) other cardiac pathology.

Comparison with referring pathologist opinion

A sample of 200 consecutive cases of SCD referred from March 2007 onwards were examined to find out whether the referring pathologists had performed pathological examination of the heart and provided a potential cardiac cause of SCD. Cardiac diagnoses were compared between the referring pathologist and M.N.S. using the kappa (κ) coefficient.

Statistical methods

Data were analysed using the statistical software Stata version 10.1 (Statacorp). Categorical data were presented as percentages and differences between groups, including changes, over time were assessed with the use of the χ^2 or Fisher's exact test. The kappa (κ) coefficient was used as a measure of agreement. Numerical data were presented as means $\pm$ SD or as median [interquartile range (IQR)]. A value $P < 0.05$ was considered statistically significant.

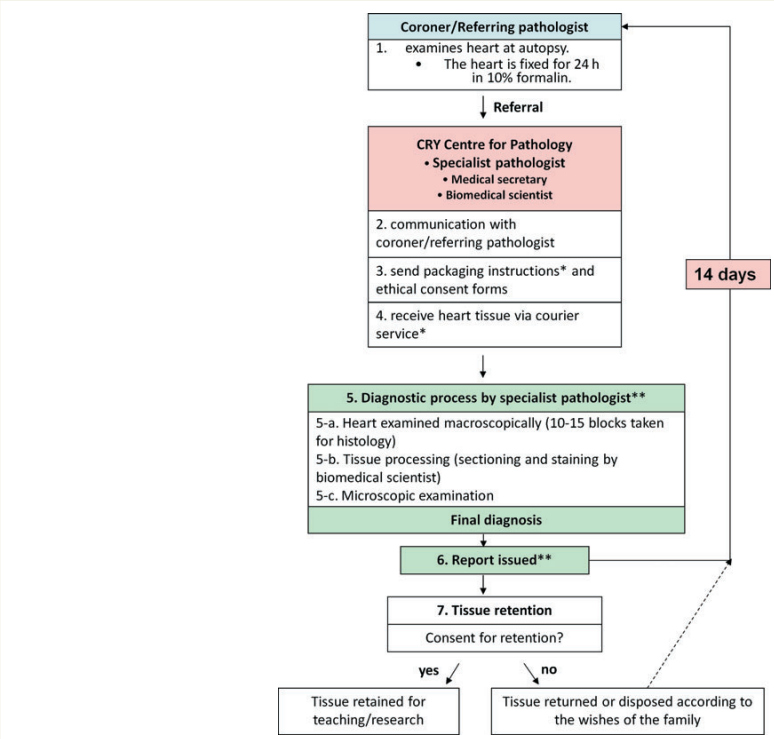

Figure 1 Flow chart showing the cardiac referral procedure at CRY CCP.
*According to national guidelines for transporting biological material.
**Free service.

Results

Referral patterns

A total of 720 cases of SCD were included in the period from March 2007 to December 2009. There was a progressive increase in the number of SCD referrals over time (*Figure 2*), with a statistically significant upward trend when comparing the periods before and after the CRY CCP launch ($P = 0.014$).

Demographics

The cohort was predominantly male ($n = 475$, 66%) and young. The median age was 32 years, age range <1–98 years, and 58% were ≤35 years of age (*Figure 3*).

Location and circumstance of death

The majority of deaths occurred at home (57%) (see Supplementary material online, *Table S2*) of whom most died at rest ($n = 244$, 34%), including those found dead in bed ($n = 197$, 27%). Sudden cardiac death occurred during or immediately after exertion in 14% of the total cohort, most of whom were young with a median age of 24 years. Sudden cardiac death in the community occurred in 144 (20%) cases, with 66 of the144 (46%) occurring during exertion, mostly on sport pitches and in leisure centres. Subjects with a

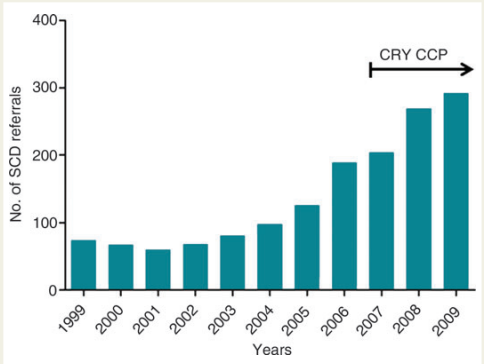

Figure 2 Bar chart depicting a rising referral pattern of SCD cases to CRY CCP and RBH over a period of 10 years. Arrow indicates the launch of CRY CCP service.

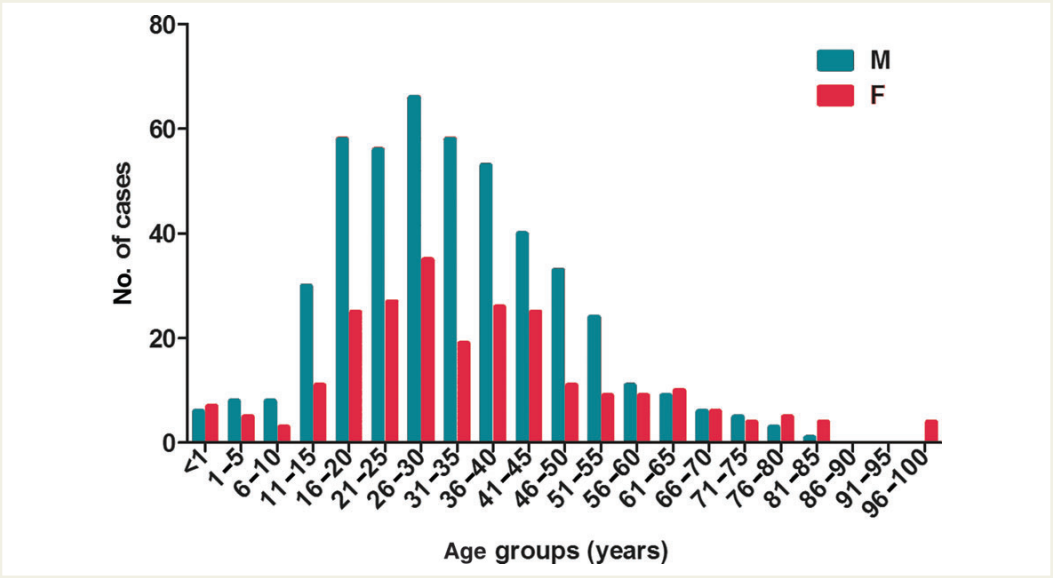

Figure 3 The demographics are shown in 720 referrals of SCD according to age and gender.

normal heart were more likely to die at rest whereas a higher proportion of deaths in individuals with a cardiomyopathy were related to exertion ($P = 0.0121$) (see Supplementary material online, *Table S3*).

Causes of sudden cardiac death

The main causes of SCD and their representation in the cohort are presented in *Tables 1*, *2*, and Supplementary material online, *Figure S1*. The most common finding was a morphologically normal heart implying SADS ($n = 321$, 45%), which was also the leading cause in those aged $\leq$35 years (228 of 422, 54%). Although males predominated (197 of 321, 61%), female SCD victims were proportionately more likely to have a normal heart (females; 51% vs. males; 41%, $P = 0.019$). The group with a morphologically normal heart was also significantly younger compared with cases with structurally abnormal hearts ($P < 0.0001$): median age as 28 years (IQR 20, 38) compared with 36 years (IQR 26, 47).

Just under one-third of the cohort ($n = 207$) had cardiomyopathy. Males were proportionately more commonly affected by cardiomyopathy than females. The most common cardiomyopathies included: idiopathic left ventricular hypertrophy (ILVH) (26%), of which 42% were associated with fibrosis; hypertrophic cardiomyopathy (HCM) (20%); arrhythmogenic right ventricular cardiomyopathy (ARVC) (14%); and obesity cardiomyopathy (14%).

Coronary artery pathology was the third main cause of death identified in 10% of all subjects. The main aetiology was atheroma (56 of 71, 79%, mean age 49.8 $\pm$ 18.1 years) of whom 13 were $\leq$35 years including an 11-year-old with known familial hypercholesterolaemia. Non-atheromatous causes occurred in younger individuals (15 of 71, 21%, mean age 36.2 $\pm$ 13.5 years) and included myocardial infarction with normal coronary arteries ($n = 7$); anomalous origin of the coronary artery ($n = 5$); right coronary artery from left coronary sinus ($n = 3$); and left coronary artery and left anterior descending artery (LAD) from the pulmonary trunk ($n = 2$); coronary artery bridging of the LAD ($n = 1$); and spontaneous coronary dissection found only in two females.

Myocardial inflammation was noted in 4% of the patients. The inflammatory types were: lymphocytic ($n = 9$), toxic ($n = 8$), eosinophillic and lymphocytic ($n = 5$), granulomatous cardiac sarcoid ($n = 4$), neutrophillic ($n = 3$), eosinophillic ($n = 2$), and acute rheumatic fever ($n = 1$).

Valvular pathology totalled 24 cases (3%) with a predominance of mitral valve prolapse (14of 24, 58%), which was associated with LV fibrosis in 11 cases, followed by bicuspid aortic valve ($n = 7$).

There were 23 cases of SCD with CHD, of which 17 (74%) had surgical correction for their conditions in early life. Mild to extensive fibrosis of the LV and/or RV was detected in the majority of CHD cases (14 of 23, 61%). Aortic dissection or rupture accounted for 13 (2%) sudden deaths that were mostly in the young males.

With the exception of two atrioventricular (AV) nodal tumours, examination of the conduction system did not establish accessory pathways, fibrosis, or inflammation within the AV nodal tissue.

Overall, there were 587 of 720 cases (80%): 321 normal hearts, 207 cardiomyopathy, 23 complex CHD, 24 valve disease, and 12 aortic dissections, where death could be attributed to a potentially genetic disorder and therefore may require evaluation of the family.

Specialist cardiac pathology compared with coronial pathology

From a sample of 200 consecutive cases examined by M.N.S., in 158 (79%), a provisional diagnosis had been made by the referring pathologist. This matched the diagnosis of M.N.S. in only 94 of 158

Table 1 Cardiac causes of SCD stratified by age

Cardiac cause of death	*n*	%	Median age (IQR)	Age range, years	≤35 years *n*	≤35 years %	>35 *n*	>35 %	*P* value[a]
Normal heart	321	45	28 (20, 38)	<1–82	228	54	93	31	<0.0001
Cardiomyopathy	207	29	36 (26, 47)	<1–98	97	23	110	36	<0.0001
Idiopathic left ventricular hypertrophy	54	8	25 (34, 39)	4–69	30	7	24	8	–
Obesity CM	29	4	30 (40, 47)	9–64	10	2	19	6	–
Hypertrophic cardiomyopathy	42	6	28 (37, 52)	7–98	19	5	23	8	–
Arrythmogenic right ventricular CM	29	4	30 (37, 46)	10–56	12	3	17	6	–
Idiopathic fibrosis	17	2	34 (26, 42)	<1–51	9	2	8	3	–
Dilated cardiomyopathy	17	2	38 (21, 49)	15–81	8	2	9	3	–
Other CM	9	1	48 (11, 55)	<1–71	4	1	5	2	–
CM NOS	10	1	35 (27, 46)	14–64	5	1	5	2	–
Coronary artery pathology	71	10	45 (34, 63)	11–82	21	5	50	17	<0.0001
Atheroma	56	8	46 (37, 66)	11–82	13	3	43	14	–
Non-atheromatous	15	2	35 (23, 45)	16–63	8	2	7	2	–
Inflammation	32	4	21 (14, 36)	<1–67	24	5	8	3	–
Complex congenital heart disease	23	3	23 (16, 30)	<1–44	19	5	4	1	–
Valvular disease	24	3	33 (23, 45)	12–79	14	3	10	3	–
Aortic disease	13	2	34 (34, 43)	13–59	8	2	5	2	–
Tumour	5	1	25 (22, 40)	7–43	3	1	2	1	–
Hypertensive heart disease	12	2	48 (38, 61)	35–85	1	0	11	4	–
Other cardiac pathology[b]	12	2	23 (18, 85)	<1–98	6	1	6	2	–
Total	720	100	32 (22, 43)	<1–98	422	100	298	100	–

CM, cardiomyopathy; NOS, not otherwise specified.
Decimal values were rounded to the nearest whole number for ease of interpretation.
Values are expressed as percentage (number/total number) or median (range).
[a]Statistical analysis of cardiac causes of sudden death by age groups ≤35 and >35 years. Statistical analysis was not performed in groups with a small number of cases.
[b]Other miscellaneous causes were: non-toxic alcohol/drug-related myocardial damage ($n = 4$), amyloid ($n = 2$), transplant rejection due to coronary allograft vasculopathy ($n = 1$), post-chemotherapy fatty replacement of the LV ($n = 1$), cardiac rupture linked to a fatty RV ($n = 1$), subendocardial fibroelastosis ($n = 2$), and arrhythmia (supraventricular tachycardia) related ischaemic damage ($n = 1$).

(59%) of the cases (*Table 3*). The κ coefficient as a statistical measure of concordance was moderately significant at 0.48. Referring pathologists were more inclined to diagnose pathology rather than designate the heart as morphologically normal with only 50 out of 80 (63%) normal hearts being described as such. Of the 30 normal hearts diagnosed with pathology by the referring pathologist, 20 were thought to have signs of cardiomyopathy, the majority being ARVC. In contrast, seven of the hearts described as normal by the referring pathologist, were in fact found to have signs of cardiac disease. Indeed, the diagnosis of ARVC was overestimated (21 vs. 5), with agreement in only 2 cases. The main observation misattributed to ARVC was isolated fatty infiltration of the RV ($n = 10$). Similarly, HCM was over-diagnosed in 10 cases with over-interpretation of myocyte disarray by the referring pathologist often due to the sampling of the anteroseptal and posteroseptal walls where myocyte disarray is a normal finding. Remaining cases were in fact ARVC ($n = 2$), non-compaction cardiomyopathy ($n = 1$), hypertensive heart disease ($n = 1$), and normal heart ($n = 1$). Myocarditis (5 of 9, 56%) was also over-reported by the referring pathologist. In the majority of cases, inflammation was focal with no myocyte necrosis. Histological images of these common confounders are shown in Supplementary material online, *Figure S2*. Consensus was greatest, however, in the diagnosis of dilated cardiomyopathy (6 of 8, 75%). In two out of five (40%) cases of valvular disease, over-interpretation of floppy mitral was noted, especially in older patients where slight ballooning of the mitral leaflet edges is a normal finding. Finally, coronary artery atheroma was considered a significant cause of death in three cases but was determined to be non-significant by M.N.S. due to over-interpretation of collapsed coronary arteries at autopsy.

Discussion

This study reports the results of a unique specialist cardiac pathology service dedicated to the pathological investigation of predominantly young SCD. Eighty per cent of deaths had pathological evidence to support potentially inherited cardiac diseases including Marfan's syndrome, cardiomyopathy, and a morphologically normally heart (SADS). Evaluation of first-degree relatives of victims with SADS identifies inherited heart diseases in up to half of the families.[1,4,5,18] It is therefore vital that SADS deaths and other deaths due to potentially inherited heart diseases are recognized accurately at post-mortem to trigger this process. Cardiac familial investigation is, however, a time-consuming and expensive process that can be distressing to relatives. For these reasons, clinicians need accurate

Table 2 Cardiac causes of sudden death by gender

	Female		Male		
Cardiac cause of death	***n***	**%**	***n***	**%**	***P* value[a]**
Normal heart	124	51	197	41	0.019
Cardiomyopathy	48	20	159	33	<0.0001
Idiopathic left ventricular hypertrophy	6	2	48	10	–
Obesity CM	12	5	17	4	–
Hypertrophic cardiomyopathy	8	3	34	7	–
Arrythmogenic right ventricular CM	2	1	27	6	–
Idiopathic fibrosis	11	4	6	1	–
Dilated cardiomyopathy	5	2	12	3	–
Other CM	3	1	6	1	–
CM NOS	1	0	9	2	–
Coronary artery pathology	23	9	48	10	0.759
Atheroma	16	7	40	8	–
Non-atheromatous	7	3	8	2	–
Inflammation	10	4	22	5	–
Complex congenital heart disease	9	4	14	3	–
Valvular disease	12	5	12	3	–
Aortic disease	3	1	10	2	–
Tumour	3	1	2	0	–
Hypertensive heart disease	7	3	5	1	–
Other cardiac pathology	6	2	6	1	–
Total	245	100	475	100	–

CM, cardiomyopathy; NOS, not otherwise specified.
Values are expressed as percentage (number/total number).
[a]Statistical analysis of cardiac causes of sudden death by gender. Statistical analysis was not performed in groups with a small number of cases.

diagnostic pathology and the confidence that the autopsy was sufficiently thorough to exclude non-genetic causes.

It is, however, as important to ascertain the absence of disease, as it is to find it. Our study demonstrates that a morphologically normal heart was in fact under-diagnosed and cardiomyopathy was over-diagnosed by general pathologists. This explained most of the incongruence (59%) in diagnoses given by the referring pathologists and the specialist cardiac pathologist. The discrepancy also supports the need for an expert cardiac pathologist to support general pathologists in the evaluation of sudden death, particularly in the young. It emphasizes the need for recognition of what is normal and abnormal, both macroscopically and microscopically. The spectrum of normal variation can be misinterpreted as pathological, such as fatty infiltration of the RV and slight ballooning of the mitral valve, both associated with the female gender and older age.[19,20]

The importance of a specialist cardiac pathology service

Erroneous autopsy interpretation may mislead clinicians and cause missed diagnostic opportunities that could result in further tragedies within the same family. This is particularly pertinent to primary care physicians and cardiologists who are required to interpret a post-mortem report, often without any guidance, as well as initiate referral of family members and plan cardiological evaluation. Our study thus highlights the importance of an experienced cardiac pathology service dedicated to providing a thorough cardiac autopsy and ensuring a higher probability of accurate interpretation.

Pathological findings and their implications

In the under 35 age group, 54% were normal heart/SADS cases. At autopsy, it is a diagnosis of exclusion where the heart is structurally normal and toxicology is negative. This is a higher proportion than other recent series, where proportions of structurally normal hearts in similar-aged groups of sudden death victims range from 26 to 43%.[21–24] This may reflect referral bias (see limitations), but nonetheless reinforces the importance of normal heart/SADS and its genetic implications.[1,4]

Among the cardiomyopathies, ILVH without the evidence of disarray was the most common structural abnormality in our cohort. Idiopathic LVH is an increasingly recognized entity in cases of SCD in athletic[25] and non-athletic individuals.[26] Although its exact significance remains unclear, a recent study from our group suggested a number of plausible hypotheses including innocent bystander, pathological variant of physiological LVH in genetically predisposed individuals, part of the HCM spectrum, and a trigger of arrhythmia in the context of an inherited arrhythmogenic syndrome.[26] Other processes can mimic inherited cardiomyopathies. For example, ARVC was over-diagnosed by coroners' pathologists based upon RV fatty infiltration alone, a common finding in an increasingly obese population and in subjects with a history of alcohol misuse or inherited myopathies. Variable amounts of intramyocardial fat in the RV have also been documented in individuals dying of non-cardiac causes.[20] The presence of fibrosis in association with fat is necessary to diagnose ARVC, reinforcing the need for detailed histological examination including the taking of further blocks by an experienced cardiac pathologist.[27]

Gender and risk of sudden cardiac death

Male gender accounted for two-thirds of SCDs in our cohort. This observation is consistent with existing literature which unanimously reports a male predominance in SCD, in both athletic and sedentary individuals.[22,28,29] This also correlates with the higher incidence of male SCDs observed in cardiomyopathies in general and in arrhythmia syndromes such as long QT 1 (LQT1) (prepubescent males), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome.[29–32] Female gender, on the other hand, has only been associated with a higher risk in long QT syndrome, particularly drug-induced torsade-de-pointes LQT2 in young female adults.[32]

Causes of sudden cardiac death by circumstance of death

Normal hearts occurred twice as often as cardiomyopathy in SCD under rest with a comparative tendency for cardiomyopathy to be more associated with SCD under exertion. This is in line with other studies. Individuals that die during sleep commonly have a

Table 3 Inter-agreement in pathologists' individual diagnoses in 158 cases

Agreement in cardiac diagnoses											
	Referring pathologist opinion										
Expert opinion	ARVC	HCM	LVH	DCM	CM NOS	Other CM	Inflammation	Valvular disease	Normal heart	Other pathology	Total
ARVC	2	2	1	0	0	0	0	0	0	0	5
HCM	0	7	1	0	0	0	0	0	1	2	11
LVH	1	5	9	0	0	0	0	0	1	0	16
DCM	0	0	0	6	0	0	0	0	0	2	8
CM NOS	0	0	0	0	4	0	0	0	0	0	4
Other CM	3	1	1	0	0	6	1	0	2	1	15
Inflammation	0	0	1	0	0	0	4	0	0	0	5
Valvular disease	0	0	0	0	0	0	0	3	1	1	5
Normal	13	1	2	0	1	3	4	2	50	4	80
Other pathology	2	1	0	0	0	1	0	0	2	3	9
Total	21	17	15	6	5	10	9	5	57	13	158

ARVC, arrhythmogenic right ventricular cardiomyopathy; CM NOS, cardiomyopathy not otherwise specified; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; other CM, other cardiomyopathy.
Shaded boxes indicate those cases where there was agreement.

normal heart and are likely to be affected by circadian rhythm and autonomic nervous system factors.[33] Sudden cardiac death in athletes during or shortly after exertion is mostly attributed to an underlying cardiomyopathy, where adrenergic surges are thought to be important.[28] Arrhythmia syndromes such as LQT1 and CPVT that are likely to underlie morphologically normal heart SCD are also associated with sudden death on exertion.[32] Others such as LQT3 and Brugada syndrome are associated with death during sleep or at rest. This is supported by the study of Behr *et al.*,[1] where most of the SADS victims died at rest/during sleep.[32]

Limitations

The CRY CCP offers a nationally recognized service and is more likely to attract challenging cases or cases where a morphologically normal heart is suspected. It does not, however, receive all SCD referrals in the country. For these reasons, conclusions drawn from this study, with particular reference to the prevalence of different causes of SCD, may not reflect the whole of the UK because of selection bias. The aim of this study was, however, to investigate the importance of expert opinion in the diagnosis of SCD. It is plausible that some referring pathologists intended to send the heart for specialist review from the outset and performed only limited histopathological evaluation. Discrepancies between the autopsy conclusions of our specialist centre and that of local pathologists may have been overestimated.

The over-diagnosis of ARVC by referring pathologists may, in part, be explained by the use of older contemporaneous task force criteria that proposed fatty replacement alone as indicative of ARVC disease. New guidelines emphasize that histology must confirm the presence of fibrosis, alone or in combination with fatty infiltration.[34] Our centre adhered to the new criteria throughout the study, long before their official acknowledgement.[7]

The CRY CCP service is based upon analysis undertaken by a single pathologist, albeit an acknowledged international expert. Having an additional pathologist to corroborate the cardiac pathological interpretation would be desirable, but single centres cannot justify more than one pathologist doing cardiac work full-time. There are also few cardiac pathology specialists in the UK, posing a limitation on double reporting. This issue is being addressed by the UK Cardiac Pathology Network[35] by establishing cardiac pathology pathways in the country to support and train general pathologists.

The use of imaging has been proposed to replace the conventional autopsy and was not included in our study. It is clear, however, from our experience that these are currently unsuitable for the accurate diagnosis of cardiac causes of death.[36]

Finally, we acknowledge the lack of post-mortem genetic testing, 'molecular autopsy', to support the diagnosis of possible genetic conditions.[1] Although genetic testing can be a useful tool, accurate conventional pathological findings remain the cornerstone of the diagnosis of conditions predisposing to SCD and subsequent guidance of familial evaluation and genetic analysis.

Conclusions

This study demonstrates the value of specialist cardiac pathological analysis in SCD, particularly in the young, and highlights that pathological diagnoses consistent with underlying cardiac genetic disease may account for a majority of cases. Accurate and reliable diagnoses

are therefore invaluable for coroners' pathologists, primary care, cardiologists, and ultimately the deceased's families. The large and increasing number of referrals to the CRY CCP reflects a demand for fast-track expert cardiac diagnostic service for SCD in the UK. We propose that this should be considered an ideal model for future service provision in the UK and abroad.

Supplementary material

Supplementary material is available at *Europace* online.

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Contributors: The concept for this study originated from M.N.S. S.V.N., J.W. and M.N.S. extracted the data. S.V.N, M.N.S., M.P., K.O., E.R.B. and S.S. analysed the data with data management support provided by W.B. All the authors contributed to the design, the interpretation of the results, drafting, critical revision of the manuscript for important intellectual content, and approved the final version of the manuscript. Additionally, M.N.S. performed the post-mortems. M.N.S. is the guarantor.

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Ethical approval

Brompton, Harefield and National Heart and Lung Institute: Ref 07/Q040.

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Low Prevalence of Risk Markers in Cases of Sudden Death Due to Brugada Syndrome

Relevance to Risk Stratification in Brugada Syndrome

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Objectives The objective of this study was to determine the prevalence of conventional risk factors in sudden arrhythmic death syndrome (SADS) probands with Brugada syndrome (BrS).

Background Patients with BrS and previous aborted sudden cardiac death (SCD) are at high risk of recurrent events. Other universally accepted clinical features associated with higher risk include unheralded syncope and the presence of a spontaneous type 1 electrocardiogram (ECG).

Methods We analyzed reported symptoms and reviewed ECGs from SADS probands with familial diagnoses of BrS, established by cardiological evaluation, including ECG, 2-dimensional echocardiography, Holter monitoring, exercise tolerance testing, and ajmaline provocation. These cases underwent familial evaluation between 2003 and 2010.

Results A total of 49 consecutive families with a confirmed SADS death and a diagnosis of BrS were evaluated, comprising assessment of 202 family members in total. One family had 2 members with SADS, resulting in a total of 50 probands included. Mean age of death of probands was 29.1 ± 10.6 years, with 41 males (82%) ($p < 0.05$). Antemortem ECGs were available for 5 SADS probands, 1 of which demonstrated a spontaneous type 1 pattern. In 45 probands, symptoms before death were reported reliably by family members. Of these, 9 (20%) had experienced at least 1 syncopal episode before the fatal event. Importantly, 68% of probands would not have fulfilled any current criteria for consideration of implantable cardioverter-defibrillator.

Conclusions The "low-risk" asymptomatic BrS group comprises the majority of SCD in this cohort. Current risk stratification would appear to be inadequate, and new markers of risk are vital. (J Am Coll Cardiol 2011;57:2340–5)

Brugada syndrome (BrS) is a primary electrical disease that predisposes those affected to life-threatening ventricular arrhythmias, which are predominantly nocturnal (1). Despite its recent introduction as a clinical entity (2), BrS has an established genetic etiology related predominantly to cardiac sodium channel dysfunction in 20% of cases and demonstrates an autosomal dominant inheritance pattern (3). Nevertheless, BrS remains characterized by stereotypical electrocardiographic (ECG) findings in affected patients (1), in combination with clinical or familial history. However, in some patients, ECG changes can fluctuate between normal and the Brugada pattern (4); hence, provocation testing with a class I antidysrhythmic, such as ajmaline, is used to unmask the BrS phenotype in suspected cases (5–8). Patients with BrS who have survived a ventricular fibrillation arrest are recommended to receive an implantable cardioverter-defibrillator (ICD) in light of the significant risk of recurrent events (1,9). The other recognized high-risk group recommended for an ICD consists of patients with symptoms secondary to a presumed self-terminating malignant arrhythmia in the presence of a type 1 Brugada ECG (10,11). A number of studies have evaluated the additional value of specialist ECG and invasive assessments

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in risk stratification, although conclusions regarding their impact have been inconsistent (9–13). We report on a retrospectively analyzed cohort of individuals who experienced unexplained sudden death, with a diagnosis of BrS established following familial evaluation (14,15), with regard to the prevalence of these high-risk characteristics.

Methods

Study cohort. This study represents unselected, consecutive, familial diagnoses of BrS from 3 tertiary referral centers in the United Kingdom, in the context of unexplained familial sudden cardiac death (SCD). Data from clinical evaluations of the blood relatives of individuals who experienced sudden arrhythmic death syndrome (SADS) referred between 2003 and 2010 to specialist inherited cardiac disease clinics at Lewisham University, King's College, and St. George's Hospitals (London) were retrospectively reviewed. All SADS probands with at least 1 blood relative diagnosed with BrS were included.

Cardiological evaluation. An evaluation protocol for families with a member with SADS has been established (Fig. 1), with particular emphasis on evaluation for evidence of familial BrS. All family members were investigated with clinical history and noninvasive evaluation by ECG, transthoracic echocardiography (including close evaluation of the right ventricle), Holter ambulatory monitoring, and stress testing, with additional magnetic resonance imaging to exclude structural heart disease.

Genetic testing. Following appropriate genetic counseling, we offered sodium channel, voltage-gated, type V, alpha subunit (SCN5A) mutation analysis to family members with a clinical diagnosis of BrS.

Ajmaline provocation test. Ajmaline provocation was performed with 1 mg/kg intravenous ajmaline administered over 5 min, with real-time ECG monitoring and ECGs recorded for analysis at 10- to 30-s intervals for 15 min from the start of ajmaline administration or until return of the ECG to baseline. Before March 2006, standard 12-lead ECG monitoring was used during ajmaline provocation.

Abbreviations and Acronyms

- **BrS** = Brugada syndrome
- **ECG** = electrocardiogram
- **ICD** = implantable cardioverter-defibrillator
- **SADS** = sudden arrhythmic death syndrome
- **SCD** = sudden cardiac death
- **SCN5A** = sodium channel, voltage-gated, type V, alpha subunit

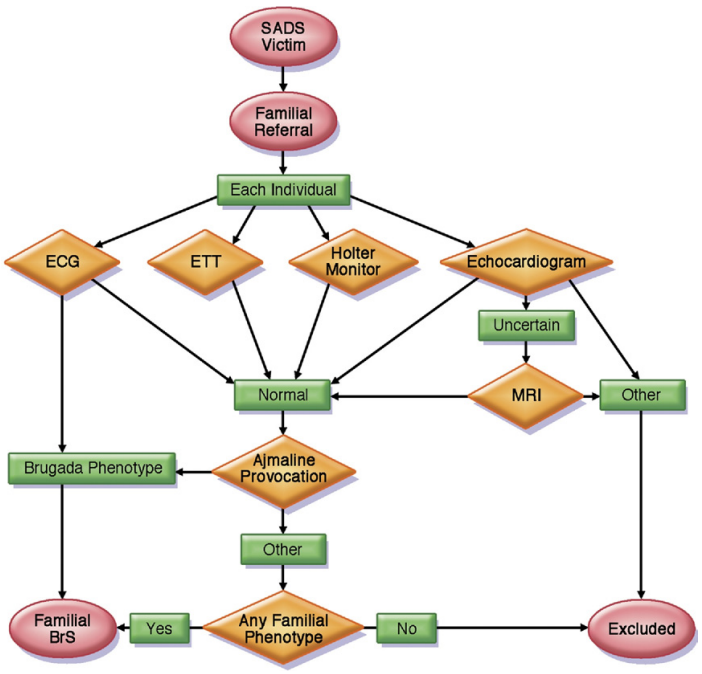

Figure 1 Investigational Cascade for SADS Probands and Their Families

A summary of the investigation undertaken following referral of a family of an individual who experienced sudden arrhythmic death syndrome (SADS) and criteria for establishing a familial diagnosis of Brugada syndrome (BrS). ECG = electrocardiogram; ETT = exercise tolerance test; MRI = magnetic resonance imaging.

Subsequently, further "high" right ventricular leads were added to improve diagnostic yield by using a 15-lead ECG recording machine. Initially, this technique used V_1, V_2, and V_3 cranially displaced by 1 intercostal space (16) in addition to the standard 12 conventional leads. In September 2009, the standard V_3 position was sacrificed in favor of including V_1 and V_2 cranially displaced in both the second and third intercostal spaces, in addition to conventional V_1 and V_2, thereby maintaining 6 right ventricular leads for diagnostic purposes. Diagnosis of a Brugada type 1 ECG pattern, either during peak ajmaline effect or at baseline, was established by ECG review by 2 investigators (H.R. and M.P.). Any individual's diagnosis of BrS was established as described in the Definitions section.

Characteristics of SADS probands. Prior familial SCD and presence of symptomatic events in each proband were determined by interviews with all evaluated family members by at least 1 of the investigators (H.R., M.P., S.S., and E.R.B.) and review of medical examiner and coroner reports. Structured clinical questions regarding the presence of prior transient loss of consciousness, seizures, or faints were retrospectively coded as probable syncopal events for the study analysis. All decisions regarding relevance of symptoms described were made by 2 investigators (H.R. and M.P.), with disputed results adjudicated by a senior investigator (E.R.B. or S.S.).

The presence of an antemortem ECG for all probands was sought by detailed questioning of evaluated family members. This included review of history of attendance at health screening events, any hospital attendance, or presence of any prior cardiovascular symptoms (palpitations or chest pain) that may have prompted an ECG. When any family members suggested the SADS proband may have attended for medical assessment prior to his or her death, the existence of an antemortem ECG was questioned by written communication to any medical professional involved in the proband's investigation. As with familial ECGs, all ECGs of probands taken before death were reviewed by 2 investigators for evidence of a spontaneous Brugada pattern.

Definitions. SADS is an umbrella term for unexpected and unexplained sudden death. It is characterized by the following conditions: sudden death; age 1 to 64 years; last seen alive and well within 12 h of being found dead; no prior recorded cardiac disease; and normal coroner's postmortem, negative toxicology results, and normal expert cardiac pathologist's examination, when available (17). A proband represents any individual who experienced a SADS death.

A type 1 Brugada ECG pattern is defined as ≥2-mm coved-type ST elevation with or without right bundle branch block pattern in at least 2 right precordial or "high" right precordial (i.e., in the second or third intercostal space) leads (18–21).

Familial diagnosis of BrS was established by the identification of spontaneous or ajmaline-provoked Brugada type 1 ECG pattern in any one family member of a SADS proband. The cause of death in the proband was presumed to be BrS-related in all cases described, following a familial diagnosis of BrS. When the familial SADS proband died at age 45 years or older, the presence of other syndromic diagnostic criteria, such as more than 1 family member with typical Brugada ECG phenotype or syncopal symptoms, was determined by additional structured questioning and review of familial cardiological evaluation results.

Results

At least 1 member of a family group was diagnosed with BrS in 49 families affected by a SADS death, suggesting that BrS is the likely etiology for any associated proband's sudden death. A total of 50 probands were included, with 1 family having 2 individuals with confirmed SADS. In 2 families reviewed, the proband was older than 45 years. One of these 2 families had stereotypical Brugada ECG changes in more than 1 blood relative, thereby fulfilling the consensus statement diagnostic criteria (1).

Demographics. Details of associated familial evaluation are provided in Table 1. In total, 202 blood relatives of probands were cardiologically evaluated and contributed to the reported proband histories. Demographic characteristics and reported symptoms in the included probands are summarized in Table 2. The mean age of death of probands was 29.1 ± 10.6 years (range 4 to 56 years). A predominance of male BrS deaths was noted (41 male [82%] vs. 9 female [18%]; $p < 0.05$). Circumstances of death were obtained for 46 probands. Of these, 18 deaths (39%) occurred during sleep, with a further 19 (41%) at rest during the daytime; only 5 (11%) occurred during or immediately after significant exertion.

Genetic testing. Details of families for whom SCN5A mutation analysis was undertaken (n = 28) are given in Table 2. Of the 5 families with unequivocal mutations, 3 have mutations that have previously been reported as disease causing (2 families with E1784K and 1 family with I1377V mutations) and 2 have highly probable novel mutations (D349H and H558fs). Overall, unequivocal mutations have been found in 18% of families for whom SCN5A mutation analysis was undertaken.

Risk profile of probands. Antemortem ECGs were available for 5 probands (Fig. 2), 1 of which demonstrated a spontaneous type 1 pattern (Fig. 2A) and was taken during presentation with gastrointestinal symptoms in a previously asymptomatic individual. A further proband had evidence of

Table 1 Breakdown of SADS Familial Evaluation

	No. of Family Members
Total evaluated	202
Mean no. evaluated per family ± SD	4.0 ± 2.4
Total diagnosed with BrS	83
Mean no. diagnosed with BrS per family ± SD	1.7 ± 1.1

BrS = Brugada syndrome; SADS = sudden arrhythmic death syndrome.

Table 2 Clinical Characteristics of SADS Probands With Familial Diagnosis of BrS

Clinical Presentation	Syncope	Asymptomatic	Unknown
No. of probands	9	36	5
Male/female	5/4	31/5	5/0
Age, yrs	29 ± 16	29 ± 10	31 ± 6
Type 1 BrS pattern/no. of ECGs available	0/2	1/3	0/0
Family history of prior SCD	1 (11)	6 (17)	0 (0)
Died in sleep or rest	8 (89)	28 (78)	1 (20)
Definite mutation/SCN5A analysis	1/5	4/22	1/1

Values are n, mean ± SD, or n (%).
ECG = electrocardiogram; SCD = sudden cardiac death; SCN5A = sodium channel, voltage-gated, type V, alpha subunit; other abbreviations as in Table 1.

a prior resting type 3 Brugada pattern in just 1 right ventricular lead (Fig. 2B). Both of these ECGs were taken more than 1 year before each proband's terminal event. None of the probands had undergone prior provocation testing for investigation of inducible Brugada ECG pattern or invasive electrophysiological assessment; none had a pre-established personal or familial diagnosis of BrS or other inherited cardiac disease. Probands' symptoms before death were reported reliably by family members in 45 cases, with the remainder uncertain of any prior medical history or symptoms. Only 9 of these 45 probands (20%) were reported to have experienced at least 1 syncopal episode before the fatal event. Seven probands (14%) had a prior family history of premature SCD, 1 of whom also had a personal history of syncope. Fifteen probands (30%) had either a prior family history of SCD or personal reported history of syncope. Among those who were previously symptomatic, 5 probands were male, whereas 4 were female.

Discussion

We report on 50 individuals who experienced sudden death related to BrS, who were diagnosed retrospectively following careful cardiological evaluation of family members. In keeping with previous reports, there was a male preponderance among probands, a significant minority with identified disease-causing familial SCN5A mutations, and deaths occurring predominantly at rest or during sleep (3,12,22).

Markers of risk in BrS. Current data regarding prospective risk stratification in patients with BrS have predominantly been determined on the basis of short- and medium-term prospective cohort observation of those identified in life. The FINGER (France, Italy, the Netherlands, Germany) study remains the largest cohort studied thus far, with 1,029 consecutive patients and indicates that a prior cardiac arrest, spontaneous type 1 ECG, and syncope were the only independent indicators of arrhythmic risk in patients with Brugada ECG (13).

In our cohort of SADS probands with BrS, only 18% (9 of 50) had a confirmed prior identified syncopal event, as determined by reported symptoms and medical history from relatives. This suggests that the majority of sudden deaths in

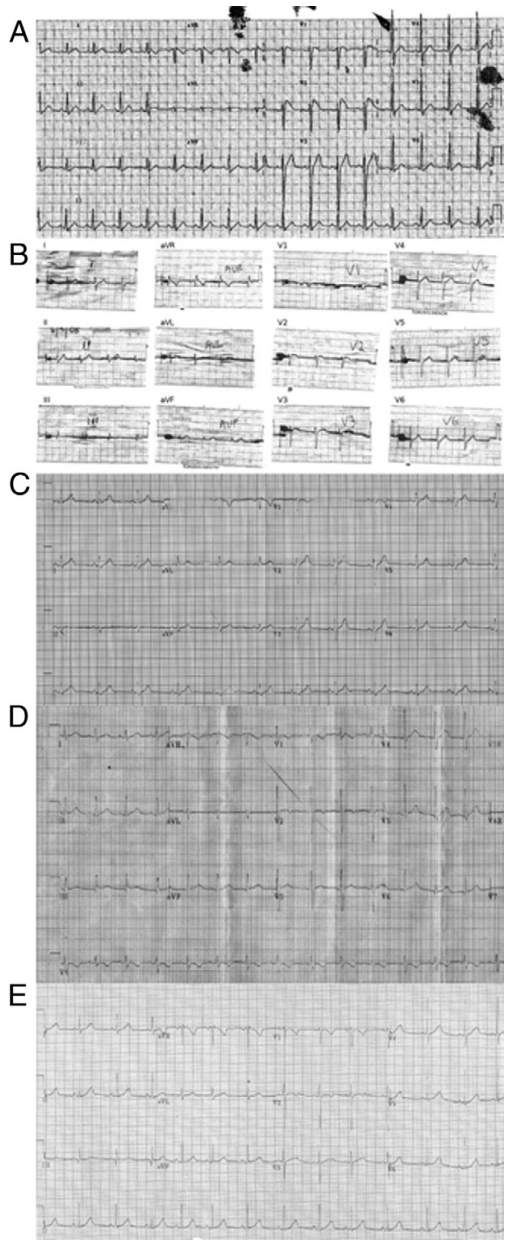

Figure 2 Antemortem ECGs in 5 SADS Probands With Brugada Syndrome

(A) The sole spontaneous type 1 antemortem ECG seen in our cohort of SADS probands with BrS. **(B)** An antemortem borderline type 3 Brugada phenotype seen in V_2 only. **(C)** A nondiagnostic antemortem adult ECG. **(D)** An antemortem ECG taken at age 8 years, with no spontaneous Brugada phenotype. **(E)** An antemortem ECG taken at age 4 years, with no spontaneous Brugada phenotype. Abbreviations as in Figure 1.

BrS occurred in asymptomatic individuals. The absence of symptoms, however, does not necessarily ensure absence of significant prior arrhythmia. Cohort observation of ICD interrogations in 19 patients with BrS with prior aborted sudden death revealed 64 episodes detected as ventricular fibrillation, 26 of which were asymptomatic by virtue of them being nocturnal and self-limiting, requiring no device discharge (1). This evidently limits the sensitivity of reported symptoms as a marker of prior ventricular arrhythmias. Furthermore, the specificity of syncope for ventricular arrhythmias among patients with BrS may be limited by the observation that there is a preponderance of other etiologies of syncope, including reports of significant vasovagal responses with head-up tilt testing among patients with BrS (23).

Although ECGs were available for a minority of probands, only 1 demonstrated a spontaneous type 1 pattern, calling into question the utility of its absence as a marker of low risk.

Of the total cohort, only 18% were identified as fulfilling 2005 consensus (1) criteria for ICD implantation on the basis of prior syncope. A further 14% may have warranted risk stratification with electrophysiological study according to consensus criteria because of the presence of a type 1 ECG before death (1 of 50) or a family history of prior SCD (6 of 50). Hence, current markers of risk for cardiac events and sudden death would have been insensitive, with 68% of our cohort categorized as low risk. Therefore, these markers may not have predicted the BrS deaths, even if a diagnosis of BrS had already been established. In particular, the majority of our cohort was asymptomatic before unheralded sudden death. Current data suggest that these asymptomatic individuals' risk would have been low, <1% per year (13), even if a spontaneous type 1 ECG pattern was seen. Given that current treatment is limited to ICD implantation, with its inherent complications in young patients, risk stratification in asymptomatic patients clearly requires improvement.

Case inclusion. The diagnosis of BrS was based primarily on the demonstration of a positive ajmaline provocation test using high right ventricular leads. Subsequent genetic evaluation of our cohort identified disease-causing SCN5A mutations in 18% of our included families. This figure is not dissimilar to that reported in previous genetic series in patients with BrS and supports our diagnostic assumption that in the context of a death from SADS, a positive ajmaline provocation test using high right ventricular leads is a genuine reflection of an underlying sodium ion channel disorder (3). The utility of high right ventricular leads has been confirmed in a few small series, predominantly in Southeast Asian patients (21,24,25). The 2005 Heart Rhythm Society and European Heart Rhythm Association consensus document recommends that this group be treated no differently than those with ECG changes in standard leads (1).

Study limitations. Despite being the largest cohort of its kind reported, this study remains limited by the relatively small number of SADS probands with BrS included. Only 5 probands had a documented prior ECG in this study. Hence, it is difficult to make judgments on the presence or absence of a spontaneous type 1 Brugada ECG in the absence of prior investigation. This is an important consideration, given its apparent importance in risk stratification (1), although syncope is a much more significant risk factor (13). Unsurprisingly, in light of their predominantly asymptomatic status, none of the SADS probands had undergone comprehensive cardiological evaluation before the terminal event. It is also possible that probands may not have relayed any prior symptoms to family members and medical practitioners.

Conclusions

This cohort suggests that the majority of individuals experiencing BrS sudden death are asymptomatic before their terminal event. Antemortem ECGs, when available, do not demonstrate a spontaneous type 1 ECG pattern. This suggests that current risk factors in BrS are insufficient to foretell unheralded sudden death events in those affected. This questions the utility of current risk stratification criteria.

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Key Words: Brugada ■ inherited cardiac conditions ■ risk stratification ■ SADS.

Sudden Cardiac Death With Autopsy Findings of Uncertain Significance

Potential for Erroneous Interpretation

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Background—The sudden death of young individuals is commonly attributed to inherited cardiac disorders, and familial evaluation is advocated. The identification of pathognomonic histopathologic findings, or the absence of cardiac pathology (sudden arrhythmic death syndrome [SADS]) at postmortem, directs familial evaluation targeting structural disorders or primary arrhythmogenic syndromes, respectively. In a proportion of autopsies, structural abnormalities of uncertain significance are reported. We explored the hypothesis that such sudden cardiac deaths represent SADS.

Methods and Results—Families (n=340) of index cases of sudden cardiac deaths who underwent postmortem evaluation were evaluated in specialist cardiogenetics clinics. Families in whom the deceased exhibited structural abnormalities of uncertain significance (n=41), such as ventricular hypertrophy, myocardial fibrosis, and minor coronary artery disease, were included in the study. Results were compared with 163 families with normal postmortem (SADS). Relatives underwent comprehensive cardiac evaluation. Twenty-one families (51%) with autopsy findings of uncertain significance received a diagnosis based on the identification of an inherited cardiac condition phenotype in ≥1 relatives: 14 Brugada syndrome; 4 long-QT syndrome; 1 catecholaminergic polymorphic ventricular tachycardia; and 2 cardiomyopathy. A similar proportion of families (47.2%) received a diagnosis in the SADS cohort (P=0.727). An arrhythmogenic syndrome was the predominant diagnosis in both cohorts (46% versus 45%; P=0.863).

Conclusions—Familial evaluation after sudden cardiac deaths with autopsy findings of uncertain significance identified a similar proportion of primary arrhythmogenic syndromes to a contemporary series of SADS. Our study highlights the need for accurate interpretation of autopsy findings to avoid erroneous diagnoses, with potentially devastating implications. **(*Circ Arrhythm Electrophysiol*. 2013;6:588-596.)**

Key Words: cardiac arrhythmia ■ cardiomyopathies ■ death, sudden cardiac ■ pathology ■ ion channel

The majority of sudden cardiac deaths (SCDs) are attributable to atherosclerotic coronary artery disease and are manifest in the older population, whereas cardiomyopathies predominate in the young (<35 years).[1] In a proportion of SCDs, a cardiac abnormality cannot be identified despite detailed histopathologic examination and toxicology screen; such cases are classified as sudden arrhythmic death syndrome (SADS).[1] The recognition of SADS is imperative, because evaluation of blood relatives of the deceased identifies a hereditary arrhythmogenic syndrome in ≈50% of families, thereby providing a likely cause of death and identifying surviving relatives at risk from the same fate.[2,3]

Clinical Perspective on p 596

The interpretation of the results of postmortem evaluation of SCD cases is a complex task and uncertainty may exist about the causal relationship between the pathological findings and the sudden death.[4] The significance of myxoid degeneration of the mitral valve with prolapse, stable atherosclerotic coronary plaque with limited (<50%) luminal stenosis and focal myocarditis, which are relatively prevalent in the general population, may be erroneously overestimated. Not infrequently, postmortem diagnoses of hypertrophic cardiomyopathy (HCM) and arrhythmogenic right ventricular cardiomyopathy are based solely on the presence of left ventricular hypertrophy (LVH) and fatty infiltration of the right ventricular wall, respectively, in the absence of pathognomonic histological changes. Left ventricular hypertrophy, however, is a recognized feature of

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physiological adaptation to exercise,[5] and fatty infiltration of the right ventricle is commonly present in obese individuals.[6] The distinction between pathology and normal variants may, therefore, be challenging in the context of SCDs.

This study explored the hypothesis that a proportion of SCDs with autopsy findings of uncertain significance may represent part of the SADS spectrum and, in particular, inherited arrhythmogenic syndromes.

Methods

Setting

The SCDs of several young individuals prompted the United Kingdom government to commission the 8th chapter of the National Service Framework for heart disease, aimed at facilitating early identification of individuals at risk of SCD. St George's Hospital and University Hospital Lewisham (London, UK) have implemented dedicated inherited cardiac diseases clinics, serving relatives of individuals who experienced SCD, from throughout the United Kingdom. Family members undergo comprehensive cardiac evaluation aimed at identifying those at risk and preventing further tragedies.

Study Cohort

Between 2003 and 2009, 368 families of cases of premature SCDs (aged between 4 and 64 years) were evaluated in our inherited cardiac diseases clinics. Criteria for inclusion in the study comprised the following: (1) unexpected death of an apparently healthy individual; (2) death from natural causes; (3) last seen alive and well within 12 hours; (4) complete postmortem report; (5) the absence of an extracardiac cause of death; and (6) negative toxicology screen. Twenty-eight families were excluded from further analysis based on the absence of a complete postmortem report (n=4), positive toxicology (n=14), and the presence of documented past medical history before death (n=10).

Postmortem reports of the 340 SCD cases were scrutinized by 2 authors and divided into the following 3 groups: Group 1, autopsy findings highly suggestive of structural cardiac pathology accounting for the SCD (n=136); Group 2, no identifiable structural cardiac pathology, consistent with an SADS death (n=163); and Group 3, autopsy findings with structural abnormalities of uncertain causal effect (n=41). In cases of disagreement a third, senior author was consulted. The main study cohort consisted of 41 families, comprising 157 blood relatives, where the postmortem report was classified into group 3. The 163 families in group 2 (SADS cohort) were used as controls for comparison (Figure 1).

Autopsy Evaluation

All cases of SCDs included in the study had undergone a full coroners' pathologist postmortem, and in 39% of cases a specialist cardiac pathologist had performed additional assessment. The diagnostic criteria for specific structural cardiac diseases and examples of autopsy findings of uncertain significance are outlined in Table 1.

Familial Cardiological Evaluation

All relatives underwent comprehensive cardiac evaluation according to a previously published protocol.[5] Baseline ECG, echocardiography, holter monitoring, and exercise tolerance testing were performed routinely. Ajmaline provocation testing to identify the type-1 Brugada phenotype was performed in the event of normal ECG recordings and echocardiograms or in the presence of type-2 or type-3 Brugada ECG patterns. Ajmaline testing was performed by placing leads V1 and V2 in the conventional 4th intercostal space as well as the higher 3rd and 2nd intercostal spaces.[7] Ajmaline testing was not performed in relatives ≤16 years of age (n=28) who did not have sinister cardiac symptoms or in patients (n=7) who refused consent.

Cardiac MRI (CMR) with gadolinium was performed in all relatives with ECG or echocardiographic features suggestive of cardiomyopathy. All relatives diagnosed with an arrhythmogenic syndrome where the deceased's postmortem findings could be interpreted to

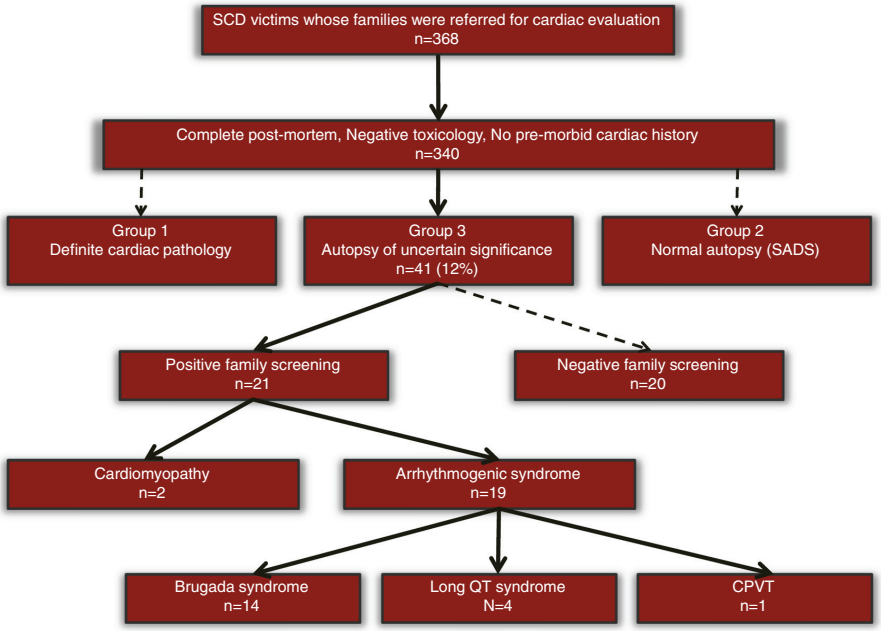

Figure 1. Flow chart of study cohort (solid arrows), including diagnostic yield. CPVT indicates catecholaminergic polymorphic ventricular tachycardia; SADS, sudden arrhythmic death syndrome; and SCD, sudden cardiac death.

Table 1. Pathological Criteria for Defining Cardiac Pathology and Certainty of Causal Effect in Sudden Cardiac Death Autopsies

Post-mortem findings highly suggestive of causal effect		Post-mortem findings of uncertain significance
Hypertrophic cardiomyopathy		
Macroscopic Left ventricular wall thickness ≥15 mm and/or heart weight ≥500 g	Microscopic Myocyte hypertrophy + disarray + interstitial fibrosis +/- abnormal intra-myocardial small vessels	Left ventricular hypertrophy and/or myocardial fibrosis in the absence of myocardial disarray
Arrhythmogenic right ventricular cardiomyopathy		
Macroscopic Right ventricular thinning + fatty replacement + fibrosis	Microscopic Fat + fibrosis of the wall of the right and/or left ventricle	Fatty infiltration of the right ventricular wall in the absence of fibrosis
Dilated cardiomyopathy		
Macroscopic Heavy heart with dilated ventricles and absence of coronary artery disease	Microscopic Absence of inflammatory myocardial disease	Mild ventricular dilatation in the absence of significant fibrosis or myocardial inflammation
Coronary atherosclerosis		
Macroscopic Atherosclerosis with estimated luminal narrowing >75%	Microscopic Acute or chronic infarction in the left ventricle	Atherosclerosis with estimated ≤50% luminal narrowing of the coronary arteries or 2mm probe patent in the absence of acute or chronic infarction
Myocarditis		
Macroscopic Normal or dilated ventricles	Microscopic Inflammation with myocyte necrosis	Scattered lymphocytic inflammatory foci with no fibrosis or myocyte necrosis
Mitral valve papillary muscle or chordae tendineae rupture with marked ballooning of both leaflets above the atrioventricular junction		Floppy mitral valve with mild ballooning between chordae in one or both leaflets
Aortic stenosis with left ventricular hypertrophy		Isolated bicuspid aortic valve

represent a cardiomyopathy (LVH, myocardial fibrosis, ventricular dilatation, and fatty infiltration of the myocardium) also underwent CMR. Further investigations were based on clinical need.

Genetic Testing

Mutation analysis was offered to all relatives with phenotypic abnormalities suggestive of inherited arrhythmogenic syndromes or cardiomyopathies, after appropriate counseling.[8] After consent, targeted mutation analysis was performed in 1 phenotypically affected member of each family, dependent on the suspected clinical condition: KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in long-QT syndrome (LQTS); SCN5A in Brugada syndrome (BrS); selected exons (7–9, 13–16, 43–50, 82–84, and 87–105) of ryanodine receptor 2 gene (RYR2) in catecholaminergic polymorphic ventricular tachycardia (CPVT). Exons and flanking intronic regions were amplified from genomic DNA, and bidirectionally sequenced to identify coding variants. Variants were labeled as pathogenic if they were previously reported to be associated with disease susceptibility[9,10]; in-frame or frameshift-causing insertions or deletions; affecting splice sites; missense mutations likely to be pathogenic, as identified by 2 in silico models (affect protein function by a tolerance index score of <0.05 in sorting intolerant from tolerant [SIFT] and classified probably damaging by polymorphism phenotyping [PolyPhen]).[11,12] If a pathogenic mutation was identified in a phenotypically affected member, other family members were offered cascade screening. Segregation analysis was used to confirm mutation pathogenicity.

Pathogenesis of SCD

An inherited condition was deemed the most likely cause of SCD if ≥1 blood relatives of the deceased exhibited phenotypic evidence of the disease. Standard criteria for the diagnosis of LQTS were used.[13] The second consensus criteria for the diagnosis of BrS were used.[14] Only the presence of the type-1 Brugada pattern (coved ST-segment elevation ≥2 mm followed by a negative T-wave) in >1 right precordial leads, including higher intercostal leads,[7] was considered diagnostic. Standard criteria were applied for the diagnosis of CPVT.[15] Cardiomyopathies were diagnosed based on published diagnostic criteria.[16]

Statistical Analysis

Data analysis was undertaken using R version 2.15.2 (R Development Core Team). Data are expressed in mean±SD. Comparison of population proportions used Fisher exact test with Donner's adjustment as necessary to account for clustered data.

Results

Characteristics of Cases of SCDs and Blood Relatives

The characteristics of the cases of SCDs are depicted in Table 2. Of the 157 blood relatives evaluated, 48% were male, with a mean age of 33.7±17.9 years (range, 9–70 years). Almost a quarter (23%) of the evaluated relatives reported cardiac symptoms with 10% having experienced ≥1 episode of syncope in the past.

Autopsy Findings and Results of Familial Evaluation

The postmortem findings of uncertain pathological significance in the 41 cases are illustrated in Figure 2. After familial evaluation, 21 (51%) out of the 41 SCD cases were considered to have died from a definite or probable inherited cardiac disorder (Figure 1). Of the 157 relatives who underwent cardiac evaluation, 36 (23%) were diagnosed with a cardiac condition, which had not been previously identified.

Diagnosis of Arrhythmogenic Syndromes

A hereditary arrhythmogenic syndrome was diagnosed in 19 of 21 families in whom an underlying inherited cardiac condition was identified. Brugada syndrome (n=14) was the predominant diagnosis, followed by LQTS (n=4) and a single case of CPVT.

After familial evaluation, an arrhythmogenic syndrome was detected in 42% (11/26) of cases where the autopsy findings were suggestive of a possible cardiomyopathy (LVH, myocardial fibrosis, ventricular dilatation, myocardial fatty infiltration; Figure 2). In these cases, all relatives with an arrhythmogenic syndrome phenotype underwent CMR scans, in addition to standard evaluation, to exclude coexistent myocardial disease. All CMR scans were reported as normal. Of interest, in the 19 SCD cases where LVH or myocardial fibrosis was reported at postmortem, (isolated LVH: n=10; myocardial fibrosis alone: n=6, or in conjunction with LVH: n=3) evaluation of family relatives identified an arrhythmogenic syndrome in ≈50% of families (5 out of 10 cases with isolated LVH and 4 out of 9 cases with myocardial fibrosis). A cardiomyopathy was diagnosed in only 1 case in either group. In the remaining 8 (42%) cases, we were unable to identify any features of inherited cardiac pathology.

Brugada syndrome was also diagnosed in 1 of the 3 families whose proband exhibited isolated fatty infiltration of the right ventricle (Figure 3.4). One of the families where the pathologist reported marked right ventricular dilatation was subsequently diagnosed with CPVT, based on the identification of typical bidirectional ventricular tachycardia on exercise testing in 2 relatives.

Moreover, 2 out of the 6 families whose probands exhibited atheromatous disease at postmortem were diagnosed with BrS. Both probands were young, aged 28 and 34 years, respectively, and exhibited up to 50% coronary artery lesions in the left anterior descending and right coronary arteries (Figure 3.1). In 1 of the 2 families where an inflammatory infiltrate commonly attributed to myocarditis was present, BrS was diagnosed during Ajmaline provocation testing in the deceased's father. In similar fashion, 1 of the 3 families whose proband exhibited pathological features of mitral valve prolapse was subsequently diagnosed with BrS based on the presence of type-1 Brugada ECG in 2 relatives.

Diagnosis of Cardiomyopathy

Only 2 families were diagnosed with an inherited cardiomyopathy; 1 dilated cardiomyopathy and 1 HCM. The first case was of a 17-year-old boy who died in his sleep. The postmortem revealed circumferential subendocardial hemorrhage with extensive myocardial fibrosis of the left ventricle. Evaluation of his relatives revealed a dilated, globally hypokinetic left ventricle in his mother and 1 of his sisters. The second case was of a 20-year-old male who died at rest. The postmortem revealed a heavy heart (>500 g) with LVH but no evidence of myocardial fibrosis or myocyte disarray. There was no history of hypertension or regular exercise. Familial evaluation revealed asymmetrical septal hypertrophy in the context of a nondilated left ventricular cavity in his father, raising suspicion of HCM. Unfortunately, the father declined further investigations.

Table 2. Characteristics of Victims of Sudden Cardiac Death With Autopsy Findings of Uncertain Significance. A Comparison Is Made With Victims With Normal Postmortem (SADS)

	Uncertain Significance (n=41)	SADS Deaths (n=163)	*P* Value
Mean age (range, y)	29.9±14.4 (4–59)	27.6±11.1 (1–56)	0.267
Sex: male	80%	67%	0.128
Ethnicity: white	93%	91%	1.000
Mode of death			
Asleep/at rest	61%	61%	<0.001
During/postexertion	37%	17%	
Unknown	2%	22%	
Reported antecedent cardiac symptoms*	37%	27%	0.250
Syncope	15%	15%	
Previous family history of premature (<55 y) sudden cardiac death	25%	17%	0.369

*Chest pain, palpitations, shortness of breath, presyncope, syncope.

Mutation Analysis

We had the opportunity to undertake mutation analysis in relatives with phenotypes suggestive of inherited cardiac conditions in 17 out of the potential 21 families. In 2 families (1:HCM, 1:BrS), individuals declined genetic testing after counseling. In 2 LQTS families, genetic testing was performed by their local geneticist. Because of the absence of coexisting atrioventricular block, mutation analysis was not performed in the dilated cardiomyopathy family.[8] Of the 13 families with BrS who underwent genetic testing, 3 carried pathogenic SCN5A mutations (R376H, H558fs, A1680T). Pathogenic mutations were also identified in the 2 LQTS families tested (E1784K and G840R in SCN5A) and in the CPVT family (A4556T in RYR2). Four of the identified mutations are previously reported as disease-associated (SCN5A R376H, A1680T, E1784K, and RYR2 A4556T).[9,10] One novel SCN5A mutation (H558fs) is a deletion, resulting in a frameshift, whereas the other (G840R) is a missense mutation with in-silico confirmation of disease causation.[11,12] A detailed description of the 6 families with a positive genotype is tabulated in the Table in the online-only Data Supplement.

Immediate Management

All relatives affected received appropriate lifestyle modification and drug avoidance advice. Eleven patients were prescribed β-blockers and 2 angiotensin-converting enzyme inhibitors. Prophylactic cardioverter defibrillators were implanted in 5 patients: 3 BrS; 2 LQTS, and 2 LQTS patients received a pacemaker.

Comparison of Diagnostic Yield With the SADS Cohort

The SADS cohort consisted of 163 families, comprising 463 relatives. The characteristics of the SADS victims are described in Table 2. The diagnostic yield in the SADS cohort

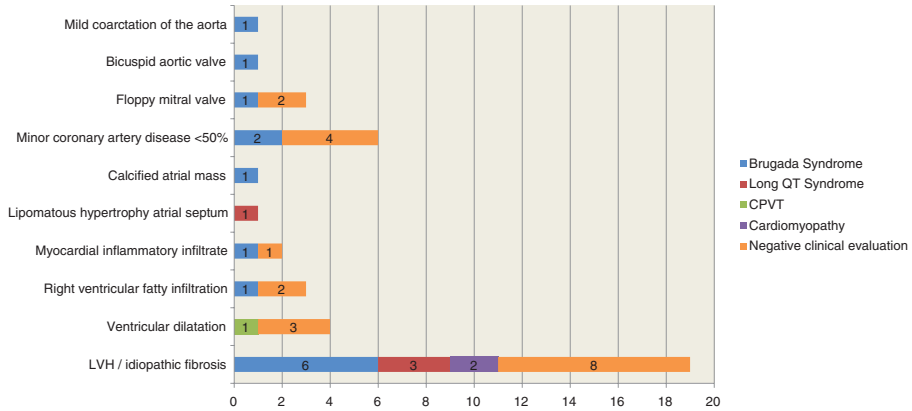

Figure 2. Histogram depicting the diagnostic yield of familial evaluation in victims of sudden cardiac death. The *x*-axis represents the number of families, and the *y*-axis represents the pathology identified in the deceased during postmortem evaluation of the heart. The different colors within the columns represent the diagnosis established after cardiac evaluation of surviving relatives with absolute numbers of families stated within the relevant color. CPVT indicates catecholaminergic polymorphic ventricular tachycardia; and LVH, left ventricular hypertrophy.

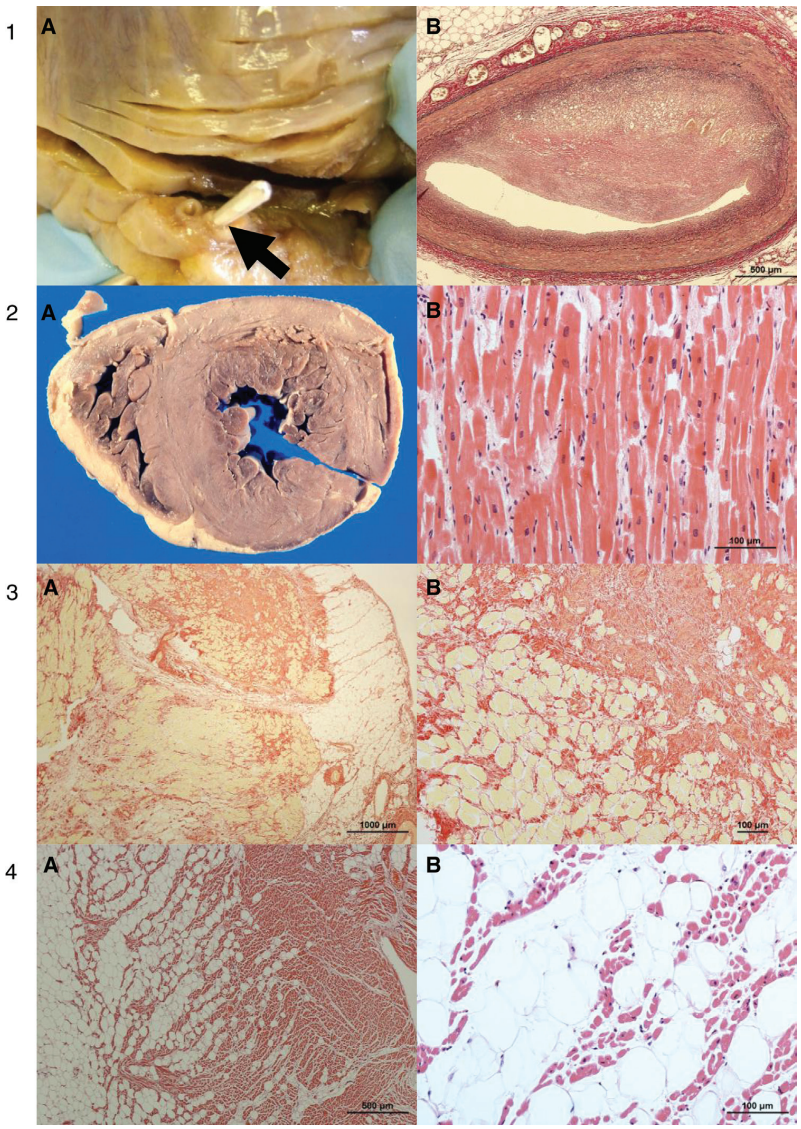

Figure 3. Histopathologic slides of: **1**, an individual who exhibited coronary artery disease on autopsy and subsequent familial evaluation identified Brugada syndrome (BrS): **A**, macroscopic examination of the left anterior descending coronary artery in an otherwise normal heart shows eccentric atheroma. This can be opened with a 2-mm probe (black arrow), indicating that there would have been normal blood flow during life; **B**, staining with Trichrome stain (Elastin Van Gieson) confirmed eccentric atheroma. **2**, An individual who exhibited isolated left ventricular hypertrophy on autopsy and subsequent familial evaluation identified long-QT syndrome: **A**, short axis slice showing a circumferentially thickened left ventricular wall measuring 2 cm. The left ventricular cavity diameter is also reduced; **B**, hematoxylin and eosin staining confirms idiopathic myocyte hypertrophy with enlarged box-shaped nuclei. No myocyte disarray is noted. **3**, An individual who exhibited myocardial fibrosis on autopsy and subsequent familial evaluation identified BrS: ×2 (**A**) and ×10 (**B**) magnification of picro-sirius red staining shows extensive myocardial replacement with collagen (stained red) in the left ventricular wall from epicardium into midmyocardium. There is also fine interstitial collagen surrounding individual myocytes (yellow). Mild fatty infiltration is also noted within the collagen areas. **4**, An individual who exhibited right ventricular fatty infiltration on autopsy and subsequent familial evaluation identified BrS: ×4 (**A**) and ×20 (**B**) magnification of hematoxylin and eosin stain of the right ventricular wall showing significant fatty infiltration in the outer third of the myocardium (stained red). There is no fibrous tissue.

was similar to that of individuals with autopsy findings of uncertain significance (47.2% versus 51%; P=0.727). In both cohorts, the predominant diagnosis was of a primary arrhythmogenic syndrome (Figure 4). A similar proportion of the relatives who were evaluated in the SADS and the autopsy findings of uncertain significance cohorts were diagnosed with a cardiac condition (24.6% versus 22.9%; P=0.715).

Discussion

SCDs in young, previously healthy individuals instigates cardiac evaluation of first-degree relatives aimed at identifying potentially inherited cardiac pathology to minimize the risk of further tragedies.[2,3] In a significant proportion of SCDs, the pathologist may observe findings that are relatively common in the general population, or findings that partially fulfill diagnostic criteria for structural cardiac disease, leaving uncertainty about causality and management of surviving relatives. In this study of 41 families with postmortem findings of uncertain significance, ≈50% were diagnosed with a hereditary arrhythmogenic syndrome, and the causes of SCD were similar to those observed in a true SADS cohort. This finding is of particular importance because by convention the absence of any cardiac pathology is considered a prerequisite for the definition of a death as SADS.[1]

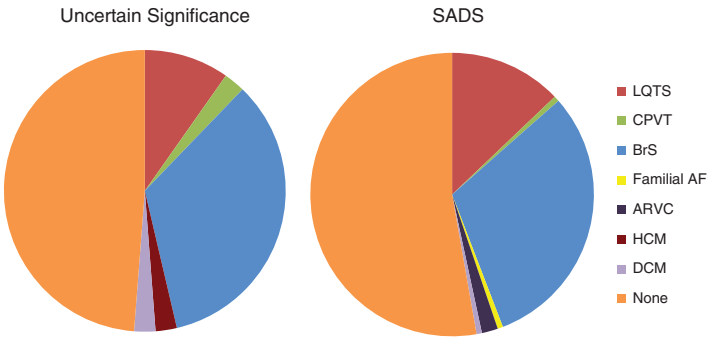

Figure 4. Pie charts depicting the results of familial evaluation in the autopsy findings of uncertain significance and the SADS cohorts. ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQTS, long-QT syndrome; and SADS, sudden arrhythmic death syndrome.

Implications of Autopsy Findings of Uncertain Significance

The causal effect of the autopsy findings is unclear. The authors offer 4 plausible hypotheses:

(a) Innocent Bystander

Bicuspid aortic valve and floppy mitral valve are present in 1% to 2% of the general population and may represent innocent bystanders. Likewise, coronary atherosclerosis without significant narrowing of the arterial lumen and without evidence of acute or chronic ischemia is common. Moreover, it is well documented that the degree of coronary artery stenosis can be overestimated by the pathologists as a result of postmortem collapse of the vessel wall.[17] Finally, foci of lymphocytes are common in the normal heart, and a degree of myocardial inflammation may be the effect of prolonged resuscitation efforts rather than evidence of myocarditis resulting in SCDs.[18]

(b) Primary Cause of SCDs

Most of the conditions identified at autopsy in our cohort have been associated with ventricular arrhythmias and sudden death.[5] Similarly, the absence of severe luminal narrowing of the coronary arteries does not preclude ventricular arrhythmias attributable to myocardial ischemia, particularly as a result of coronary artery vasospasm,[19] and isolated fatty infiltration involving the cardiac conduction system has been implicated in SCD of obese people.[20]

(c) Trigger in the Context of an Arrhythmogenic Syndrome

Consideration must also be given to the fact that structural cardiac disorders may serve as triggers for arrhythmias in the context of a coexistent inherited arrhythmogenic syndrome. One third of SCDs in our cohort with minor coronary disease were subsequently attributed to an arrhythmogenic syndrome. Current evidence suggest that the presence of coronary artery disease is an independent risk factor for LQTS-related symptomatic events.[21] It seems likely that transient ischemia alters the arrhythmic substrate by reducing the threshold for afterdepolarizations or increasing transmural dispersion of repolarization, both recognized mechanisms for arrhythmogenesis in ion-channel disease.[22]

(d) Spectrum of Arrhythmogenic Syndromes

There is mounting evidence that individuals with ion-channel defects may exhibit structural cardiac changes.[22] Although the majority of BrS patients possess a structurally normal heart, a small proportion, seems to exhibit evidence of ventricular wall motion abnormalities, ventricular dilatation, and fibrosis.[23,24] Such structural abnormalities may be subtle, requiring sophisticated diagnostic tools.[25] Several theories have been postulated to correlate ion-channel dysfunction with structural abnormalities, ranging from impaired excitation–contraction coupling and energy production, to a hibernation-like state which over time may even lead to intracellular lipid accumulation.[22] Support for potential structural abnormalities in patients with BrS is also provided by the study of Nademanee et al[26] where the authors identified the anterior aspect of the RVOT epicardium as the substrate for the Brugada ECG pattern.

Additionally, there are reports in the literature of identical mutations presenting with either a cardiomyopathy or an arrhythmogenic syndrome phenotype, suggesting that structural and ion-channel defects may be part of a spectrum incorporating myocardial disease and primary arrhythmogenic syndromes. Mutations in the cardiac ryanodine receptor gene, commonly implicated in CPVT, have been reported in individuals exhibiting an arrhythmogenic right ventricular cardiomyopathy phenotype.[27] Mutations in the SCN5A gene, implicated in BrS, may present with arrhythmia, conduction disease, and atrial or ventricular dilatation.[28] Heritable SCN5A defects have also been associated with early-onset dilated cardiomyopathy and atrial fibrillation.[29]

LVH and Myocardial Fibrosis

In our cohort, isolated LVH and myocardial fibrosis were the most prevalent findings. Idiopathic LVH is an increasingly recognized entity in cases of SCDs. It remains unclear whether it represents an innocent bystander, a pathological variant of physiological LVH in genetically predisposed individuals or part of the HCM spectrum.[5] Although LVH is a well-recognized feature of cardiovascular adaptation to exercise, in our study, only 4 out of the 10 individuals exhibiting isolated LVH exercised on a regular basis. Data from the Framingham study also indicate that LVH confers a 4-fold risk of sudden death.[30] In addition, experimental studies suggest that LVH alters ion-channel expression and function predisposing to reentry arrhythmias and ventricular fibrillation.[31] Although in the majority of individuals such adaptations are unlikely to result in increased risk of arrhythmias, the development of LVH in an

individual with an underlying arrhythmogenic syndrome may exacerbate electric instability and predispose to sudden death.

The amount of myocardial fibrosis and the collagen texture seem to play a role in vulnerability to arrhythmia.[32] Moreover, myocardial fibrosis may represent incomplete expression of underlying cardiomyopathy. Myocardial fibrosis has also been reported in marathon runners and in cases of SCDs in athletic individuals, raising concerns whether prolonged arduous exercise can lead to repeated myocardial injury, necrosis, and subsequent fibrosis.[8] Finally, animal models have demonstrated that SCN5A mutations cause progressive impairment of atrial and ventricular conduction associated with myocardial rearrangements and fibrosis.[33]

The Role of the Cardiac Pathologist

This study highlights the importance of accurate interpretation of the autopsy findings because false conclusions may misguide familial evaluation or offer false reassurance to surviving relatives and dissuade physicians from initiating familial screening. Given the relative rarity of SCDs from inherited conditions and the challenges associated with their diagnosis, the authors propose that all cases of SCDs and, particularly, SCDs in young (≤35 years) individuals, where an inherited condition is suspected or diagnostic uncertainty remains as to the cause of death, should be referred for further evaluation to an expert cardiac pathologist.

Limitations

The predominant diagnosis in our cohort was of BrS, reflecting the victims' demographics (80% males, mean age of 30 years), predominant mode of death (60% asleep/at rest), and the routine use of Ajmaline provocation testing. It is plausible that some Ajmaline-based diagnoses may be erroneous. Although currently there are no large series of normal subjects undergoing Ajmaline test, existing literature in SCN5A positive families suggests that the specificity of the Ajmaline challenge exceeds 94%.[34] Therefore, in the absence of an alternative gold standard, provocation testing with a sodium channel blocker remains an integral part of the evaluation of individuals with suspected BrS.

The authors also concede that given the relative novelty of the condition and the association of the Brugada phenotype with several structural cardiac abnormalities, it is possible that some of the relatives exhibiting the Brugada phenotype did not have a genuine arrhythmogenic syndrome. However, all individuals who were diagnosed with BrS underwent comprehensive evaluation, including a detailed echocardiogram, and a significant proportion were subjected to CMR, and none exhibited any evidence, suggesting structural cardiac anomalies. Further support for the presence of BrS is underscored by the genetic yield (23%) of pathogenic SCN5A mutations, similar to existing literature.[14]

In the 6 families in whom a pathogenic mutation was identified in evaluated relatives, we were unable to perform postmortem analysis in the tissues of the victims for confirmation of the genotype because no tissue was available by the time the relatives were evaluated in our clinic. In the United Kingdom, the Human Tissue Act does not permit retention of tissue as part of a deceased patient's record, and retention for research requires familial consent at the time of postmortem.[35] As such, in the majority of cases, histological slides are prepared, reported, and imaged at the time of the postmortem examination, allowing early return of the tissue for burial or cremation.

Conclusion

The current study underscores the need for accurate interpretation of autopsy findings in cases of SCDs to avoid erroneous diagnoses with potentially devastating implications for surviving relatives. Our data suggest that all SCDs with inconclusive autopsy findings should be considered as potential SADS deaths, and comprehensive evaluation of family relatives for both inherited primary arrhythmogenic syndromes and structural cardiac abnormalities should be advocated.

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Disclosures

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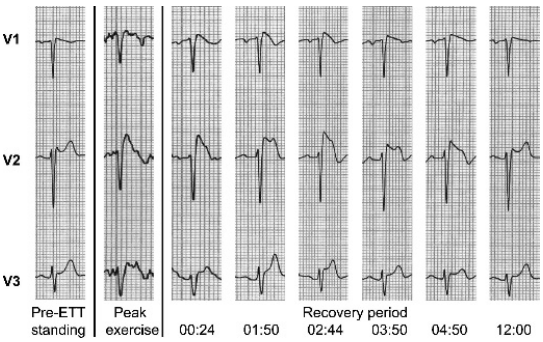
CLINICAL PERSPECTIVES

Sudden death in young individuals is commonly attributed to inherited cardiac diseases. Postmortem examination is a critical first diagnostic step to guide clinical evaluation of surviving relatives toward structural disorders or primary arrhythmogenic syndromes. In a significant proportion of sudden cardiac deaths, the pathologist may identify structural abnormalities, which are relatively prevalent in the general population or do not quite fulfill established diagnostic criteria; therefore, an element of uncertainty may exist about the causal relationship between the pathological findings and the sudden death. This is the first study to demonstrate that ≈50% of such deaths may be attributable to arrhythmogenic syndromes implicated in sudden arrhythmic death syndrome (SADS). This finding is of particular importance because conventional criteria require the absence of any cardiac pathology as a prerequisite for the definition of a death as SADS. In the current study, familial evaluation after sudden cardiac death with autopsy findings of uncertain significance identified a similar proportion of primary arrhythmogenic syndromes to a large series of SADS. Our results highlight the need for accurate interpretation of autopsy findings by physicians involved in the decision-making process before the family reaching a cardiogenetics clinic, to avoid erroneous diagnoses, or worse still, false reassurances with potentially devastating implications for surviving relatives. Our data suggest that all sudden cardiac deaths with inconclusive autopsy findings should be considered as potential SADS deaths, and comprehensive evaluation of family relatives for both inherited primary arrhythmogenic syndromes and structural cardiac abnormalities should be advocated.

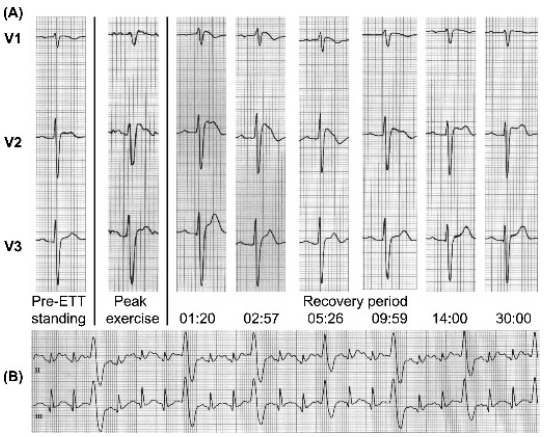
Unmasking of the Brugada phenotype during exercise testing and its association with ventricular arrhythmia on the recovery phase

Two largely asymptomatic men, a 36-year-old (patient A) and a 56-year-old (patient B), were evaluated following the sudden death of a first-degree relative. Both resting 12-lead electrocardiograms exhibited minor ST-segment elevation in V1 and 0.2 mV saddle-shaped ST-segment elevation in V2. During an exercise test, patient A developed 0.7 mV ST-segment elevation with coved pattern and T-wave inversion in V2 and ST-segment elevation and T-wave inversion in V3, at peak exertion (panel A). The test was terminated. Patient B exercised to exhaustion without significant ECG changes but exhibited multiple ventricular extrasystoles in recovery that terminated spontaneously (panel B(B)). Both patients exhibited ST-segment elevation in leads V1–V3, highly characteristic of the Brugada syndrome (panel A and panel B(A)) post exercise. Both remained asymptomatic. An Ajmaline provocation test was positive supporting a diagnosis of Brugada syndrome. Coronary angiography and cardiac magnetic resonance imaging were normal. Both patients were offered an internal cardioverter defibrillator.

Brugada syndrome predisposes to fatal arrhythmias and most deaths occur at rest.[1] Exercise is not considered a risk factor and exercise testing is not routinely performed. Scarce reports in the literature describe normalisation of the ST-segment elevation, with increased sympathetic activity during exercise and unmasking of the syndrome with increased parasympathetic tone in recovery.[2] In patient A, however, the Brugada phenotype developed during peak exertion, refuting this simplistic theory as the sole explanation. In patient B the ventricular ectopics in recovery raise concerns regarding the safety of strenuous exertion in predisposed individuals and highlight the importance of exercise testing in the diagnosis and risk stratification of Brugada syndrome.



Panel A (Patient A) ECG strips of the right precordial leads V1–V3. ST-segment changes before, at peak and during the recovery phase of the treadmill exercise stress test. ETT, exercise tolerance test.



Panel B (Patient B) (A) ECG strips of the right precordial leads V1–V3. ST-segment changes before, at peak and during the recovery phase of the treadmill exercise stress test. (B) Rhythm strip demonstrating multiple ventricular extrasystoles of increasing frequency 40 s into recovery. The ectopics terminated spontaneously within 10 s. ETT, exercise tolerance test.

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Appendix 4: List of abstract publications arising from the thesis

Papadakis M, Chandra N, Raju H, Bastiaenen R, O'Sullivan A, Fonseca T, Sawyer E, van Niekerk N, Sharma S. The diagnostic yield of Brugada syndrome in victims of sudden arrhythmic death syndrome; an underestimated entity? J Heart Disease 2010;7:79
15th World Congress of Heart Disease, Jul 2010, Vancouver, Canada

Papadakis M, Baines G, Kouloubinis A, O'sullivan A, Van Niekerk N, Chandra N, Rawlins J, Sharma S. The diagnostic yield of Brugada syndrome in families affected by sudden arrhythmic death syndrome; The impact of higher intercostal V1 and V2 leads. Heart 2009;95:33
British Cardiovascular Society annual conference, Jun 2010, Manchester, UK

Papadakis M, Edwards C, Rawlins JC, Chandra N, O'Sullivan A, Sawyer E, White T, Sharma S. Current risk stratification protocols fail to identify the majority of sudden arrhythmic death victims secondary to Brugada syndrome. Heart 2009;95:33
British Cardiovascular Society annual conference, Jun 2009, London, UK

Papadakis M, Edwards C, Rawlins JC, Sharma S. Brugada syndrome: The absence of symptoms is a poor predictor of identifying individuals at risk of sudden death. Circulation 2008;118:S_981
American Heart Association scientific sessions, Nov 2008, New Orleans, USA

Papadakis M, Chandra N, Raju H, Bastiaenen R, O'Sullivan A, Fonseca T, Sawyer E, van Niekerk N, Sharma S. The diagnostic yield of Brugada syndrome in victims of sudden

arrhythmic death syndrome; an underestimated entity? Eur Heart J 2010;31: (Abstract Supplement), 841

European Society of Cardiology congress, Aug 2010, Stockholm, Sweden

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